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VOLUME 139 *2008*

As many of our readers are aware, for the last two years we have been working on the initial stages of transforming the Journal of the Entomological Society of Ontario into a timely, modern entomological publication. Having accomplished our first tasks of resuming timely, annual publication of both the print and electronic versions, we are now moving towards several new goals. One of these is a project to create electronic versions of all JESO back issues, so that these can be made freely available on the JESO website. Another is to encourage more entomologists, professionals, students, and amateurs to consider submitting their work to JESO. JESO is especially relevant for entomologists working on the Ontario entomofauna, but we also welcome submissions from those working on insects in other regions. We also warmly encourage the submission of student research. A major goal of Entomological Society of Ontario is to promote the growth of entomology in Ontario and elsewhere, and as one member of the ESO recently put it, 'JESO is in the business of promoting research on insects', in all its many shapes and forms'.

This volume of JESO reflects our enthusiasm for research on insects, in all its many shapes and forms. The research presented in Volume 139 (2008) ranges from taxonomic and faunistic studies, to studies of the effects of insecticides and viruses on their target species. The methodologies employed range from the time-honoured, classical approaches of careful collecting, preserving, and microscope study, to molecular approaches based on analyses of DNA sequences and RNA splicing patterns. In fact, one might say that the theme of this volume is diversity, both of the the insect taxa studied, and of the types of research and research methods employed. I find this to be an especially gratifying aspect of the current volume, because in an age where entomologists are themselves becoming a rare species, it behoves us to broaden our perspective on the kind of science that qualifies as entomology.

Happy reading!

Miriam H. Richards
Editor

WINTER MANAGEMENT OPTIONS FOR THE ORCHARD POLLINATOR *OSMIA LIGNARIA* SAY (HYMENOPTERA: MEGACHILIDAE) IN NOVA SCOTIA

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Abstract

J. ent. Soc. Ont. 139: 3–18

The Blue Orchard Bee, *Osmia lignaria* Say, a commercially available solitary bee, was recently introduced into the Annapolis Valley of Nova Scotia for assessment as a manageable pollinator of apple. An important component of this assessment was to investigate options for wintering this species as few apple growers have storage facilities suitable for the recommended wintering practices. Specifically, winter survival under ambient outside, albeit sheltered conditions was compared to wintering bees in controlled environmental chambers at 4°C. In addition, a comparison of survival was made between bees from two populations; one introduced into Nova Scotia the previous year and reared for a complete generation versus one reared in Utah and imported to Nova Scotia as dormant un-emerged adults.

Populations wintered outside fared slightly but significantly better than those wintered inside, but each location proved suitable for wintering. Bees from both populations had high survival (as measured by emergence from natal cocoons), but rates were significantly greater in bees reared in Nova Scotia for one year. Overall, bees from both populations proved suitably cold hardy for wintering in Nova Scotia, as evidenced by both high rates of survival and by enhanced supercooling capacity. Wintering bees in controlled conditions offers the advantages of predictable climate, and controlled synchrony of emergence with crop flowering. However, access to and/or lack of climate controlled facilities may be a limiting factor in adopting this species for commercial use in the province. This study thus provides evidence that bees wintered outside have natural emergence coinciding favourably with apple flowering, and that placing nests in sheltered outdoor environments in Nova Scotia provides an affordable and safe approach for wintering *O. lignaria* populations for apple pollination.

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Introduction

The last few decades have witnessed many changes in the management of bees (Hymenoptera: Apoidea) for the pollination of crops in North America. One of these changes has involved a small but significant shift from reliance on one species, the ubiquitous honey bee, *Apis mellifera* L., for most of our entomophilous pollination needs. The predictions and warnings of potential problems likely to arise due to almost total reliance on honey bees for crop pollination have been realized as evidenced by the large losses in the number of colonies available for pollination (Kevan 2001; DeGrandi-Hoffman 2003) resulting in recent native pollinator conservation initiatives and literature (Matheson et al. 1996; Stubbs and Drummond 2001; Strickler and Cane 2003; Committee on the Status of Pollinators in North America 2007). In response, interest in the development of non-*Apis* bees as potential crop pollinators has increased, and several potentially useful species have been investigated (Torchio 1990b, 2003; Bosch and Kemp 1999, 2000; Cane 2005).

Some of the earliest research on non-*Apis* bees and their subsequent development as crop pollinators started in the 1940's in the USA (Torchio 1990a). In addition to the need to learn about basic bee biology and diversity, this early research was initiated because of the realization that honey bees are poor pollinators of some crops and that several indigenous species were more efficient crop pollinators (Bohart 1972). More recently, replacement crop pollinators have been sought to fill gaps left by the increased demands on an ever decreasing number of honey bee colonies, and these efforts have resulted in a growing number of non-*Apis* bees being developed for management in North America (Parker et al. 1987; Torchio 1990a; Richards 1993; Strickler and Cane 2003). However, the number of bees currently managed does not approach 1% of the estimated 3000-4000 species in America north of Mexico (Krombein et al. 1979).

The main differences between managing honey bees and all other bee species currently used for crop pollination in North America stem from differences in social complexity and differing life histories. No other species of bee exhibits levels of social behaviour as complex as that observed in the genus *Apis* (Michener 1974, 2007). In America north of Mexico, all indigenous bee species have a solitary stage in their life history, making it the world's largest land area with no social species wintering as a colony. As such, most temperate zone bee species have dormant periods corresponding to the winter season (Stephen et al. 1969).

Despite the fact that most bee species spend the largest proportion of their life cycle in a non-reproductive/non-feeding stage often overlapping the winter months, little is known about the behavioural and eco-physiological adaptations of bees leading up to and during the winter in temperate zones (Sakagami et al. 1981; Hoshikawa et al. 1992; Rust 1995). For insects in general, properties of the wintering hibernacula such as moisture levels, ground cover, nesting depth, the ground's slope and sun exposure levels are often as important as the physiological and behavioural adaptations of the insect (i.e., cryo-protectant synthesis, supercooling) for enhancing survival (Danks 1978, 1991; Leather et al. 1993). Therefore, such factors are important and must be considered when developing management techniques for solitary pollinators, as evidenced in the past by the works of Krunic and Hinks (1972), Fairey et al. (1987), and more recently by Wilson and Abel (1996), Bosch and Kemp (2000, 2003, 2004), Bosch et al. (2000), Kemp and Bosch (2005),

and Kemp et al. (2004).

Cavity-nesting megachilid bees (Megachildae) are among the forerunners for management for crop pollination (Sheffield et al. 2008) and for these species, wintering in controlled climate conditions is recommended (Fairey et al. 1987; Richards et al. 1987; Bosch and Kemp 2001, 2003, 2004). However, in Nova Scotia and elsewhere in North America, most growers do not own or have access to controlled cold storage facilities. Therefore, finding alternative methods to safely overwinter *Osmia lignaria* Say for orchard pollination is a priority for managing this species. Considering that *O. lignaria* is indigenous throughout temperate North America (Krombein et al. 1979) though not confirmed in Nova Scotia (Sheffield et al. 2003, 2008), and that its flight period naturally corresponds with flowering of many rosaceous tree fruits, it seems a reasonable assumption that this species can overwinter successfully under ambient conditions within apple producing regions.
From 2000-2004, populations of *O. lignaria* were imported into Nova Scotia for evaluation as a replacement pollinator for apple, and to develop region specific management strategies. The objective of this study was to investigate *O. lignaria* winter storage options available to Nova Scotia apple growers or groups interested in rearing this species for commercial tree fruit pollination. Specifically, comparisons in survival and winter physiology were made between *O. lignaria* from two populations wintered outside in a sheltered environment versus in a traditional cold storage facility in the winter of 2001-2002. One population consisted of the offspring of bees imported into Nova Scotia in the fall of 2000 and released for pollination trials in 2001; the second was offspring of a population imported from Utah following the active season of 2001.

Materials and Methods

Nest Preparation

Following the active adult season (late May until early July 2001), nesting blocks containing developing larvae of *O. lignaria* were placed in an unheated screened building (insectary) maintained at the Atlantic Food and Horticulture Research Centre, Kentville, Nova Scotia, to allow development to continue through to the adult stage (i.e., the wintering stage) within the natal cocoons. In early autumn, samples of randomly selected nesting tubes were removed from nesting blocks and split lengthwise to expose cocoons and determine the number and sex of bees; caution was used to ensure the tunnel end plug was left intact. Sex was determined based on cocoon size and position within the nesting tube. Each cocoon was removed in sequence from the nesting tube and brushed lightly to remove faecal particles and other debris. Undamaged cocoons were selected and weighed to 0.001g, and placed into nesting tubes with the same orientation; females positioned in the rear. Nesting tubes were then sealed longitudinally with masking tape. The same procedure was repeated on a portion of dormant bees received from Utah in the autumn of 2001, which were already split upon arrival as part of quality assessment by the supplier (Torchio Enterprises, North Logan, UT). Pre-winter weights of male and female bees from both locations were compared using Analysis of Variance (ANOVA).

Samples from the two locations were divided into 24 groups (twelve each from Nova Scotia and Utah), each group having at least 20 females among the nesting tubes. The

nesting tubes of each group were then placed into a wooden laminate nesting block with 81 tunnels (arranged in a 9 x 9 pattern), each tunnel approximately 0.85 cm in diameter and 15 cm deep (i.e., able to house the nest liners) (Fig. 1a). The nesting tubes were randomly selected and placed into the inner 49 nesting tunnels (i.e., a 7 x 7 pattern) of each nesting block until all were accounted for.

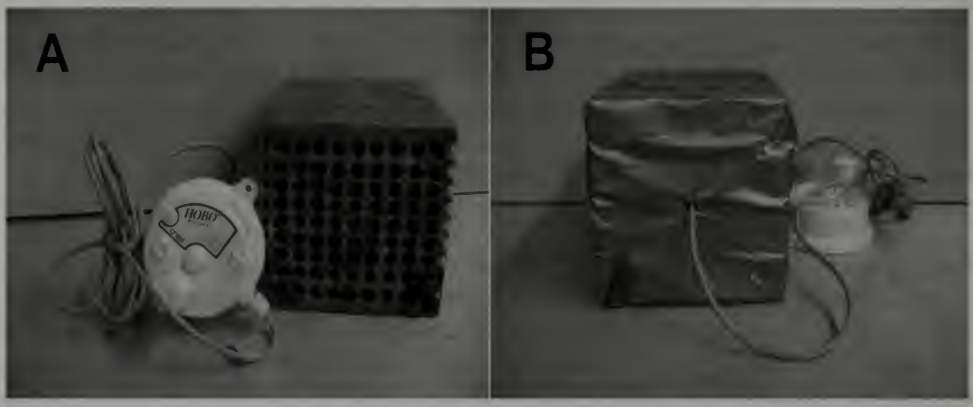

FIGURE 1. A) Nest used for *Osmia lignaria* overwintering studies, showing B) data logger probe position.

Winter Survival

The nesting blocks were randomly assigned to the two wintering conditions, resulting in six blocks from each population in each wintering site. Data loggers (Onset; HOBO7 H8 Pro Series model H08-031-08 and HOBO7 H8 4-Channel model H08-006-04) with external probes were used to record ambient temperature (°C) at each of the wintering sites (six channels per site) and within the nesting tubes (six channels per site, with three allocated to the Nova Scotia and Utah populations) (Fig. 1b).

In the spring of 2002, bees wintered inside were slowly warmed to 25°C, and emerged bees from each block were collected. Following the emergence period, nesting tubes were examined for dead un-emerged bees (i.e., adult bees which were still inside their cocoons and presumably died during wintering). Bees that had chewed out of their cocoons and died due to nesting tube blockage were included in the analysis as "live bees" as it was apparent they survived the winter. The number of cocoons containing dead larvae or pupae were subtracted from the starting total as death would have occurred prior to winter (these made up a very low proportion (<1%) of the population).

Bees wintered outside were allowed to emerge naturally to determine emergence phenology of this species in Nova Scotia. Bees emerging daily from each nesting block were collected with numbers and sex recorded. Following the emergence period, nests were examined for un-emerged adult bees as above.

This experiment was set up and analysed as a multi-factorial design; the three fixed factors of interest were 1) wintering site (inside at 4°C, 50-70% RH versus outside ambient

conditions in an unheated screened building), 2) location of origin (Nova Scotia population versus Utah population), and 3) sex. In addition, interactions between each factor and among all three factors were studied. The proportion of bees surviving for each nesting block was subject to arcsine transformation (Zar 1999) to achieve normality and homoscedacicity of variance, which was successful. All analyses were therefore conducted in the transformed form, although the reported population parameters are of non-transformed data.

Supercooling Point Determination and Weight Loss

At two intervals throughout the winter (January and March), sub-samples of cocoons were removed from the nesting blocks. These were re-weighed to determine percent weight loss within the interval of wintering. Bees were then removed from their cocoons, placed into a gelatin capsule, and were attached with petroleum jelly to a thermocouple within a NalgeneTm 2.0 ml cryovial. The cryovial was then submerged in coolant in an FTS-Systems ultra-low temperature bath (Stone Ridge, NY) interfaced with a computer using virtual instrumentation (VI) Lab View 5 (National Instruments, Austin, TX) software and hardware (Nubuss port and A/D boards). From an initial bath temperature between 10°C and 15°C, the temperature was dropped at a rate of 1°C /min (after Salt 1966), and supercooling points were measured to the nearest 0.1°C; the supercooling point is the lowest body temperature recorded prior to the increase in temperature due to the latent heat released during crystallization (Lee 1991).

Nonparametric analysis (based on ranked data) was used on both supercooling point and weight loss data sets as they were not normally distributed and variances were unequal. For non-parametric analysis of supercooling points, each datum was transformed to a positive value, T, using the following formula $T = (a°C)(-1)$, where a represents the supercooling point datum (which is always a negative number). This transformation was done as more negative supercooling points are indicative of enhanced cold-hardiness, and it allowed the most negative values to have the highest rank instead of the lowest.

Results

Overwintering Survival

Populations overwintered outside had significantly higher mean survival rates than those kept inside ($F = 16.59$; $df = 1$; $p < 0.001$), and bees reared in Nova Scotia had significantly higher mean survival rates than populations imported from Utah ($F = 29.78$; $df = 1$; $p < 0.001$) (Fig. 2). Significant interactions were observed between origin x wintering site ($F = 5.28$; $df = 1$; $p = 0.027$), and the main effects plot (Fig. 2) indicated that origin had a larger main effect than the wintering site (indicated by the slightly steeper slope). Sex did not significantly influence wintering survival ($F = 0.04$; $df = 1$; $p = 0.851$) or the two-way interactions of 'origin x sex' and 'wintering site x sex', although these did approach significance ($F = 3.15$; $df = 1$; $p = 0.083$ and $F = 3.22$; $df = 1$; $p = 0.080$, respectively). The three-way interaction of 'wintering site x origin x sex' was not significant ($F = 0.28$; $df = 1$; $p = 0.60$).

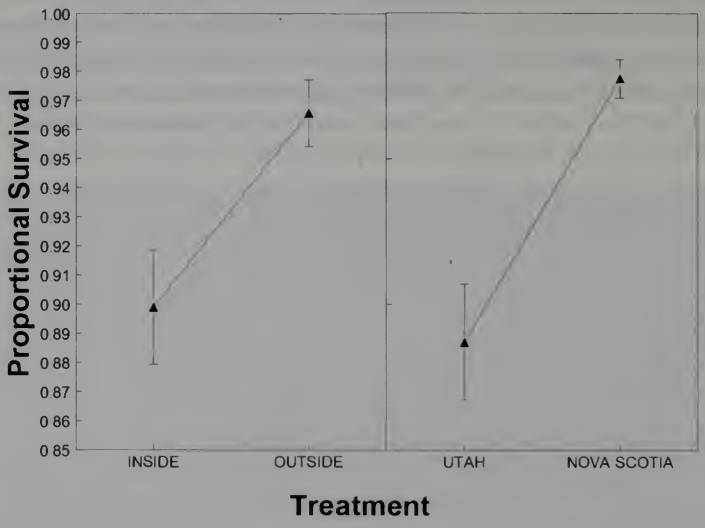

FIGURE 2. Main effects plot of mean proportional survival (± s.e.) for *Osmia lignaria* wintered inside versus outside (left graph), and from Utah versus Nova Scotia (right graph).

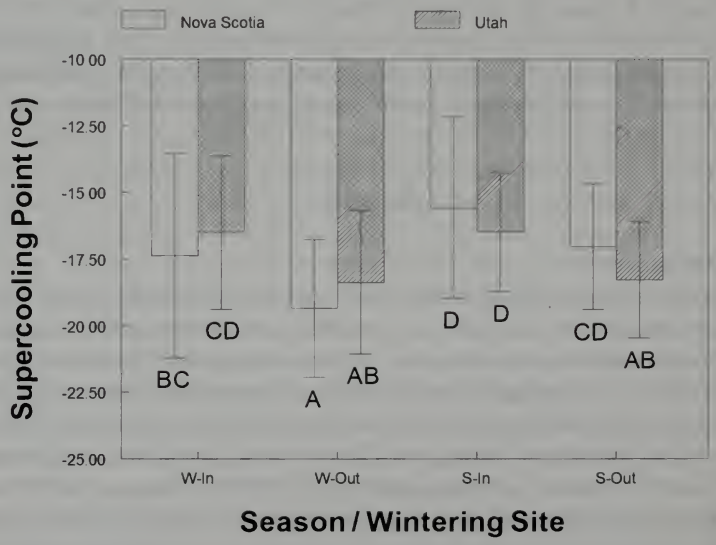

FIGURE 3. Mean supercooling points (°C ± s.e.) of Nova Scotia and Utah populations of *Osmia lignaria* wintered in environmental chambers (IN) and outside in a screened building (OUT) in the winter (W) and late winter/early spring (S). Bars sharing letters are not significantly different (ANOVA of ranked data with Tukey's HSD test; $p = 0.05$).

Supercooling

No significant differences in supercooling points were observed between sexes within any of the treatment conditions, so sex-data within treatments were pooled. Significant differences were found among the treatments ($F = 15.62$; $df = 7$; $p < 0.001$), so Tukey's HSD test was used to separate means of the ranked data (Fig. 3). Early in the winter (i.e., January), bees wintered outside (W-Out) from both locations of origin had significantly lower supercooling points than their respective counter parts wintered inside (W-In) (Fig. 3). At this time, bees from Nova Scotia-Outside had slightly lower supercooling points (mean = -19.4°C; n = 71; range = -10.1°C – -23.1°C) than those from Utah-Outside (mean = -18.4°C; n = 45; range = -9.5°C – -22.7°C), but the differences were not significant. The same trend was observed for bees wintered inside in the early winter: Nova Scotia (mean = -17.4°C; n = 52; range = -6.8°C – -22.3°C); Utah (mean = -16.5°C; n = 47; range = -7.4°C – -20.9°C).

In the spring (i.e., March) outside (S-Out) treatment, bees from Utah (mean = -18.3°C; n = 43; range = -8.3°C – -21.2°C) still had significantly lower supercooling points than those inside (S-In) (mean = -16.5°C; n = 52; range = -10.7°C – -20.2°C), but no differences were observed between the supercooling points of winter and spring bees in the respective wintering site (Fig. 3). In the spring, the supercooling points of Nova Scotia bees kept outside (mean = -17.0°C; n = 73; range = -9.6°C – -21.2°C) were lower but did not differ significantly from those inside (mean = -15.6°C; n = 54; range = -5.3°C – -19.3°C). Unlike the Utah population, bees from Nova Scotia (inside and outside) had significantly reduced cold-hardiness in spring versus the winter (Fig. 3). Only within the S-Out treatment did bees from both populations differ significantly.

Weight Loss

Significant differences were observed between the weights of bees from both populations ($F = 1011.4$; $df = 3$; $p < 0.001$), so means were separated using Tukey's HSD test ($p = 0.05$). The pre-winter weights of bees from Utah were significantly greater than bees reared in Nova Scotia (Fig. 4). Males from both populations were significantly lighter than their respective females, but Nova Scotia females were significantly heavier than Utah males (Fig. 4).

Although significant differences were observed among all treatments ($F = 4.55$; $df = 15$; $p < 0.001$), no significant differences in weight loss were observed among females and males, or between wintering sites for bees reared in Nova Scotia (Figs. 5a and b). Weight loss ranged between 1.2% - 2.1% total body wt in early winter, 1.8% - 2.6% by the spring. Similar trends were observed in bees from Utah. Weight loss in the winter ranged from 0.9% - 1.7%, overlapping the results from Nova Scotia bees. By the spring bees had lost between 2.1% - 3.3% body weight (Figs. 5c and d). Utah females wintered inside lost more weight by the spring than any other group (Tukey's HSD test; $p = 0.05$). In both populations, early winter weight loss was slightly greater (though not significantly so) in bees wintered outside than inside, while the reverse was observed in the spring (Fig. 5).

Wintering Temperatures

Temperatures inside the environmental chamber fluctuated slightly between 4°C and 6°C throughout the experiment (Dec 2001 - May 2002) with a few notable exceptions

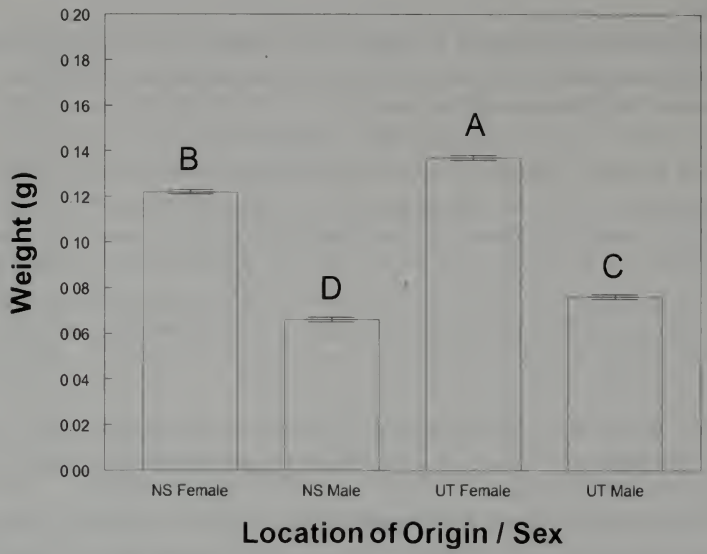

FIGURE 4. Mean pre-winter weights (g ± s.e.) of female and male *Osmia lignaria* from Nova Scotia and Utah populations. Bars sharing letters are not significantly different (Tukey's HSD test; p = 0.05; n = 250).

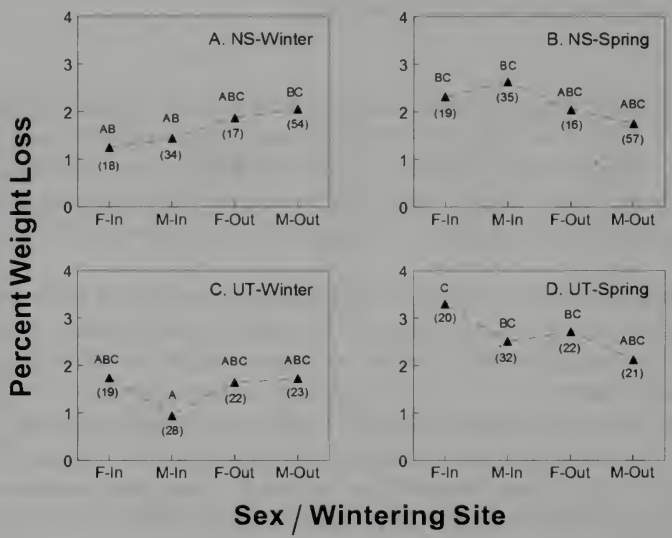

FIGURE 5. Percent weight loss during two wintering periods (A and C; Winter: Oct – early Jan; B and D; Spring: mid Jan – late Mar) in *Osmia lignaria* populations from Nova Scotia (NS) and Utah (UT) wintered in environmental chambers (In) and outside in a screened building (Out). Data markers sharing letters are not significantly different (Tukey's HSD test; p = 0.05); sample size in ( ) below each data marker.

(Fig. 6): on 12 Dec 2001 the temperature ranged between -2°C and 10°C; 07 Feb 2002 the temperature ranged between 0°C and 11°C; 16 Mar 2002 the temperature ranged between -3°C and 8°C. The corresponding temperatures recorded inside the nesting tubes had a range falling within those observed within the chamber, but followed the same trend.

Temperatures within the screened insectary (outside) showed large fluctuations between night (daily minimums) and day (daily maximums); on several instances throughout the winter a range of 20°C was observed within a 24 h period (Fig. 6). The lowest temperature recorded was below -20°C, observed on 12 Feb 2002. More than half the days in Jan-Feb 2002 stayed below 0°C; Dec 2001 and Mar 2002 had most daytime temperatures above 0°C and night time temperatures below 0°C. In Apr and May 2002, temperatures seldom fell below freezing.

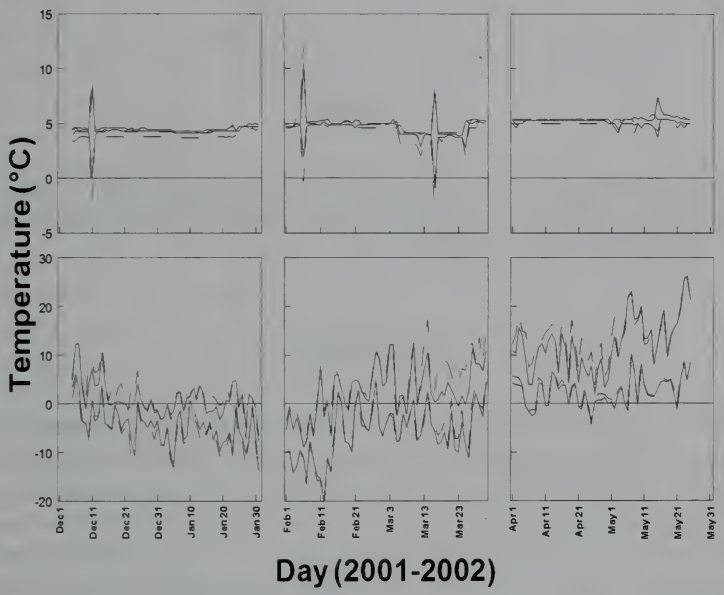

FIGURE 6. Maximum and minimum ambient air (solid lines) and nesting tunnel temperatures (dashed lines) within an environmental cabinet maintained at 4°C (top row) and within an unheated screened insectary (bottom row) from Dec 1, 2001 to May 31, 2002.

Emergence and Bloom Phenology

Bees from both populations had similar emergence patterns, although the sex ratio of bees from Nova Scotia was highly male biased (Fig. 7a). Male emergence began approximately 01 May 2002, with most individuals emerged within one week. Female emergence began approximately one week later than males, its start corresponding to the peak of male emergence. Female emergence peaked approximately ten days prior to commencement of the bloom period of McIntosh apples (Fig. 7).

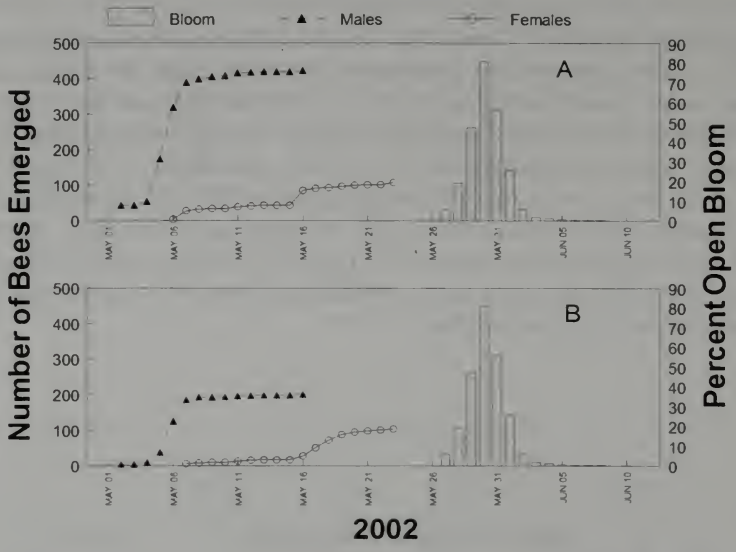

FIGURE 7. Natural emergence of male and female *Osmia lignaria* from A) Nova Scotia and B) Utah wintered in an unheated screened insectary, and the flowering phenology (percent open bloom) of McIntosh apples in 2002.

Discussion

Wintering success in insects is a complex symphony of internal physiological adaptations and behaviour modifications prior to, during and following the winter. In addition to these organism-level considerations, many ecological factors play significant roles in dictating insect survival (Danks 1978, 1991; Leather et al. 1993). Surviving the winter is therefore tied to several aspects of an insect's life history, and the most complete understanding of its ability to overwinter successfully comes from taking all of these aspects into consideration, as they do not necessarily work in isolation of one another (Danks 1978; Leather et al. 1993). In addition, understanding the complexity of wintering mechanisms which act on insect populations is critical to developing strategies for management which involve, by necessity, a wintering period.

Considering this, several factors may have contributed to the slight but significantly higher survival of bees reared in Nova Scotia than those from Utah. The first and possibly most important involved the comparative total length of the adult dormant period, in particular the pre-wintering period. In Utah, *O. lignaria* is released for apple pollination from late-April to mid-May, and adult activity usually ceases by mid-June (Torchio 1985; Bosch and Kemp 2000, 2001). However, in Nova Scotia bees are released approximately one month later as apple bloom typically occurs from late-May to mid-June (Fig. 7). In terms of development, populations reared under ambient conditions in Utah reach the adult wintering stage by August/September (Rust 1995; Bosch and Kemp 2000, 2001) which

again is earlier than in Nova Scotia. The bees from Utah were not imported into Nova Scotia until late-October/November, as bees had to be at least in the prepupal/pupal stages prior to shipment, and due to delays with importing procedures. The result was a much extended pre-winter storage period for this population compared to the one reared in Nova Scotia.

Bosch and Kemp (2003, 2004) report significant weight loss and mortality when pre-wintering duration is extensive for *Osmia lignaria* and the closely related *O. cornuta* (Latreille), and suggest an optimum range of between 10 and 30 days following adulthood for both species under commercial storage conditions. The pre-wintering period is apparently a critical one for fat body depletion, and placing bees within cold storage within the suggested time frame greatly reduces the rate of loss in *O. lignaria* (Bosch et al. 2000; Bosch and Kemp 2001, 2004).

The extended pre-wintering period also lengthened the overall wintering period for this population. Kemp et al. (2004) reported that after approximately three months of wintering under controlled conditions, *O. lignaria* enters a postdiapause transitional period, indicated in that study by increased oxygen consumption rates. Bosch and Kemp (2001, 2003, 2004) provide detailed accounts of several aspects of winter storage for *O. lignaria* and *O. cornuta*, and for the later species, maximum survival and longevity were found in populations wintered for 90-150 days (Bosch and Kemp 2004). In general, excessive prolonged storage (and/or excessive warm winter temperatures) causes additional depletion of the winter reserves (i.e., fat body) beyond a critical level, as the bees enter the postdiapause transitional period (Kemp et al. 2004). For longer storage periods, colder temperatures (i.e., 0°C) are favourable to even slightly warmer ones (i.e., 4°C) (Bosch and Kemp 2003). The depletion of these resources during the postdiapause transitional period occurs at a faster rate than during the winter, and bees held longer than one month beyond the recommend duration show increased mortality (Bosch and Kemp 2001) (bees may even become active while in cold storage at 4°C during the post diapause transitional period – some male *O. lignaria* have been observed chewing and emerging when they are held beyond the typical release time). Thus, winter survival is influenced mainly by fat body depletion via increased metabolism which can be assessed by measuring respiration rates and weight loss. A similar affect from prolonged storage has been reported in another solitary commercial pollinator, the alfalfa leafcutter bee, *Megachile rotundata* (Richards et al. 1987), although bees of the genus *Megachile* overwinter as prepupae.

Despite the extended pre-wintering/wintering period of the Utah population, survival was higher than expected (>85%) based on findings reported by Bosch and Kemp (2004), but those authors report a similar rate of mortality (16.6%) for bees held at 4°C for 210 days (Bosch and Kemp 2003). The slight discrepancy between the findings of that study (Bosch and Kemp 2004) and the present one is probably due to the standard by which survival was measured. Bosch and Kemp (2004) recognized that survival is more than just getting through the winter, and thus measured longevity of emerged bees which gives some indication of the bees ability to fly, feed and mate following the winter. In the present study, bees were considered survivors if they actually emerged, or if inspection of nesting tubes revealed bees which had chewed out of the natal cocoons even if they never emerged from the nesting tube (mainly due to obstruction).

Body size has been also indicated as a factor affecting wintering success in

O. lignaria. Tepedino and Torchio (1982) found that smaller than average offspring of both sexes of *O. lignaria* have a greater likelihood of dying during the winter, and that mortality in females (the larger sex) is higher than in comparable weighted males. In this study, variability was observed within the weight range of both sexes (Fig. 4). In a study comparing winter mortality of two distinct populations of *O. lignaria*, Rust (1995) found that mortality was significantly higher in bees from Reno, Nevada than those from Logan, Utah when both populations were wintered in the later, more northern location. The differences in mortality in that study (Rust 1995) were almost entirely attributed to the differences in winter temperatures (both average monthly temperatures and extreme minima) normally observed between the two locations. Obviously, winter temperatures are a major factor limiting the geographic ranges of many species of organisms, and local populations of a given species may show some localized differences in cold-hardiness. However, another factor might have been body size (i.e., weight) differences between males and females from both populations as per Tepedino and Torchio (1982). The significantly lighter bees (based on the reported emergence weights) from Nevada had significantly higher winter mortality than those from Utah, but in that study bees from Nevada were trap nested in tubes of 5, 6, 7, 8, and 9 mm diameter versus the uniform 7 mm tubes used in Utah (Rust 1995). Bee body size is limited in the smaller sized tubes (Tepedino and Torchio 1989), a characteristic of cavity-nesting species (Roulston and Cane 2000), and this may have contributed significantly to the mortality differences observed. Other factors such as dietary history differences (food plant type and quality) between the two populations also may have contributed to the observed body size differences, as could the time when eggs were laid; size of progeny becomes smaller as the season progresses (Tepedino and Torchio 1989). In the present study, there were significant weight differences between populations (Nova Scotia reared bees were lighter), but this did not seem to influence mortality, as the heavier Utah bees had higher mortality.

Despite the slight but significant differences in survival observed between populations, high survival was obtained under both controlled and ambient outdoor conditions (Fig. 2), even with the extended pre-wintering period in the Utah population and with minimum outside temperatures approaching the supercooling points of wintering bees. For the time being, *O. lignaria* must be imported into eastern Canada to obtain adequate numbers for apple pollination. Importation should always be done in the late summer or early autumn when bees have reached the immature non-feeding stages (i.e., prepupal and pupal), but prior to the critical pre-wintering period indicated by Bosch and Kemp (2004). Once bees have reached the adult wintering stage, exposure to elevated pre-winter temperatures should be avoided (Kemp et al. 2004) due to increased winter mortality from excessive fat body depletion (Bosch et al. 2000). Conditions during transit may be variable and temperatures above 5°C may actually increase mortality, or cause bees to emerge prematurely. Consistent or gradually fluctuating temperatures promote survival, while rapid fluctuations in daily minimum and maximum temperatures can be detrimental (Leather et al. 1993).

Bosch and Kemp (2001, 2003, 2004) stressed the importance of monitoring the populations for determining the development stages prior to wintering, a suggestion even more relevant for importation of bees into a different geographic location. For winter storage

of bees imported into Nova Scotia, the life stage of the shipment should be determined, and when possible, cocoons should be placed immediately into controlled storage facilities at 0°C. Subsequent generations reared in Nova Scotia may be kept outside; ambient temperatures in the province are suitable for winter storage as long as bees are placed in sheltered areas to avoid extreme low temperatures beyond the supercooling capacity. In general, wintering *O. lignaria* in unheated but sheltered conditions is a viable and affordable alternative to expensive controlled storage facilities for apple producers in Nova Scotia. *O. cornifrons* also showed high survival under different winter storage conditions, with comparable survival in controlled and ambient outdoor conditions in Iowa, USA (Wilson and Abel 1996).

Based on one year, emergence of *O. lignaria* populations wintered under ambient conditions in Nova Scotia corresponds favourably with the flowering period of apple (Fig. 7), as 2-3 days are required for the majority of females to mate and establish nests (Bosch and Kemp 2003). Prior to apple flowering, dandelion (*Taraxacum sp.*) serves as a main food source in Nova Scotia (Sheffield 2006). However, since mating and nest establishment would occur during pre-flowering of the crop, caution must be used with spraying to control pre-flowering pests as death of males and females within the crop system could occur. Predictable emergence and tighter synchrony with crop flowering remains the main benefit of controlled storage (Bosch and Kemp 2003).

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FLIGHT ACTIVITY AND SUSCEPTIBILITY TO INSECTICIDES OF VARIEGATED CUTWORM, *PERIDROMA SAUCIA* (HÜBNER) ATTACKING FIELD TOMOATOES IN SOUTHWESTERN ONTARIO

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Abstract

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The variegated cutworm, *Peridroma saucia* (Hübner), is a polyphagous pest that sporadically damages processing field tomatoes in southwestern Ontario. Recent anecdotal reports have suggested that it has developed resistance to pyrethroid insecticides used to control them. The objectives of this study were to acquire information on variegated cutworm flight activity in southwestern Ontario tomato fields and to assess the toxicity of currently registered and novel insecticides to determine if it has developed resistance to them.

Pheromone trap data (2006) in Norfolk County suggested 2 peaks of adult flight in field tomatoes - July and August, while in Essex County there were 3 peaks - July, August and September. Direct contact toxicity bioassays were done using larvae from a laboratory culture established at the University of Guelph in late summer 2006 from larvae collected from tomato fields in both counties. Of the 4 insecticides registered for use, lambda-cyhalothrin was most toxic to 3rd-4th instar larvae > permethrin > chlorpyrifos > methomyl. Chlorantraniliprole was most toxic of the reduced risk insecticides tested. Spinosad and metaflumizone, which act primarily as stomach poisons, were less toxic by direct contact. Second instar larvae were most susceptible to permethrin > 3rd-4th > 5th instar. Comparison of results with 1977 published data showed that variegated cutworm had developed low level resistance to methomyl but not to chlorpyrifos or permethrin. Results of the study showed that pyrethroid insecticides will be effective so long as stage of larval development and climatic conditions are considered and insecticides are applied in a manner resulting in the most effective penetration of the plant canopy as possible.

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Introduction

The variegated cutworm (VCW), *Peridroma saucia* (Hübner) (Lepidoptera: Noctuidae) is a generalist feeder on vegetable crops, cereals, ornamentals, fruit and forage crops (Rings et al. 1976). Adults migrate into southwestern Ontario and are present throughout the growing season (McClanahan and Elliot 1976). Larvae sporadically damage crops in the early part of the growing season but usually are a greater problem in mid-summer, especially in fields of processing tomatoes (Harris et al. 1977). While the organochlorine insecticides provided effective VCW control (Harris et al. 1961), organophosphorus and carbamate insecticides were less so, with effectiveness being very dependant on stage of larval development and method of insecticide application (Harris and Svec 1968; Harris and Kinoshita 1977; Harris et al. 1977). Pyrethroid insecticides provided effective VCW control (Harris et al. 1977). Insecticides recommended for cutworm control in Ontario are carbaryl (Sevin XLR), methomyl (Lannate LV), acephate (Orthene 75SP), permethrin (Pounce EC) and lambda-cyhalothrin (Matador 120EC), with the pyrethroids being considered most effective. However, recently anecdotal reports of less than adequate control have suggested that VCW has developed resistance to pyrethroid insecticides.

The objectives of this study were to: 1) monitor VCW flight in tomato fields in southwestern Ontario; 2) assess the effectiveness of currently registered and reduced risk insecticides on different larval instars; and, 3) determine whether VCW has developed resistance to presently registered control products.

Methods and Materials

Flight Activity

In Summer 2006, 6 bucket-style pheromone traps (Muti-Pher®) (2 traps/field) baited with Trece® Pherocon Cap VGC – variegated cutworm pheromone lures (Distributions Solida Inc., Saint Ferreol Les Neiges, QC) were operated from late June to mid-September at 3 tomato farms in Norfolk County. All traps were placed in hedgerows surrounding the fields. Traps were checked weekly from 26 June to 14 September and numbers of adult males captured were recorded. Pheromone lures were replaced in mid-July to maintain effectiveness. A Hercon® Vaportape II (Gempler's® – Div. of Lab Safety Supply Inc., Madison, WI) insecticide (10% dichlorvos) strip was placed in each trap to kill adults and prevent escape.

Four pheromone traps (2 traps/field) were operated from early July to late September at 2 commercial fresh-market tomato fields in Essex County. Traps were checked regularly from 11 June to 19 September and numbers of adult males captured were recorded. Two traps (1 at each location) did not operate correctly because of malfunctioning electrical generators, therefore trap catch data were generated from only 2 traps in Essex County.

Insecticide Effectiveness

Insect Culture

The laboratory colony was started in summer 2006 from late instar VCW larvae collected from tomato fields in Norfolk and Essex Counties. Larvae were reared in screened

plastic containers (34 x 25 x 13 cm) filled with 5 cm sterilized sandy loam. Chinese cabbage, *Brassica rapa* L. (Brassicacaeae), was provided as food. Larvae were fed 3 times a week and dry, rotten or moldy food materials were removed. Pupae were collected and placed in adult cages made of 11 L ice cream buckets with a screened opening (15 x 10 cm) in the side. Red Gatorade® and deionized water were used as food and moisture sources. A piece of red tissue paper (20 x 20 cm) was crumpled and placed in the container for use as an oviposition site. Pieces of tissue paper with egg masses attached were placed on 9 cm diameter filter paper in 10 cm diameter Petri dishes with a commercial VCW artificial diet (Southland Products Inc., Lake Villages, AK) cube (0.5 cm^3) Rearing cages were kept in growth chambers set at 23 ± 2°C, 60-70% RH and 16:8 L:D.

Insecticide Assays

Direct contact toxicities of technical grade (> 95% purity) permethrin (FMC Corp., Philadelphia. PA), chlorpyrifos (Dow AgroSciences Canada, Inc., Calgary, AB), methomyl (E.I. DuPont Canada Co., Mississauga, ON), lambda-cyhalothrin (Syngenta Crop Protection Canada, Inc., Guelph, ON), chlorantraniliprole (E.I. DuPont Canada Co., Mississauga, ON), , spinosad (Dow AgroSciences Canada, Inc., Calgary, AB) and metaflumizone (BASF Canada, Inc., Mississauga, ON) to VCW larvae were determined using a Potter spray tower (PST) (Potter 1959) following the procedure described by Harris et al. (1977). Primary screening tests were done with 4 concentrations of the insecticide dissolved in 19:1 acetone:olive oil. Controls treated with the solvent mixture alone were included with each insecticide. Two groups of 10, 3rd-4th instar larvae were tested at each insecticide concentration. Each bioassay was repeated 3 times resulting in 60 treated larvae per concentration. Larvae were transferred with a fine paint brush to a clean 10 cm diameter glass Petri dish lined with 9 cm diameter filter paper. Five ml of the desired concentration of each insecticide was then applied to each dish via the PST. Immediately after treatment the larvae were placed in waxed paper Dixie® cups (10/cup) filled with 1 cm sifted sandy loam and two commercial VCW artificial diet cubes (0.5 cm^3) were provided as food. Cups were covered with a 10 cm diameter glass Petri dish and were placed in a holding room at 25 ± 1°C and 16:8 L:D. Mortality counts were made after 48 h. Larvae were considered dead if unable to crawl. Second and 5th instar larvae also were tested to determine the susceptibility of the different larval stages to pyrethroids. Corrections for natural mortality (< 10%) were made using Abbott's formula (Abbott 1925).

Results

Flight Activity

In 2006, there were 2 peaks of adult activity in Norfolk County in July and August. Populations dropped to low levels in mid-August remaining that way until the study was terminated in mid-September (Fig. 1). There were 3 smaller peaks of adult activity in July, August and September in Essex County (Fig. 1).

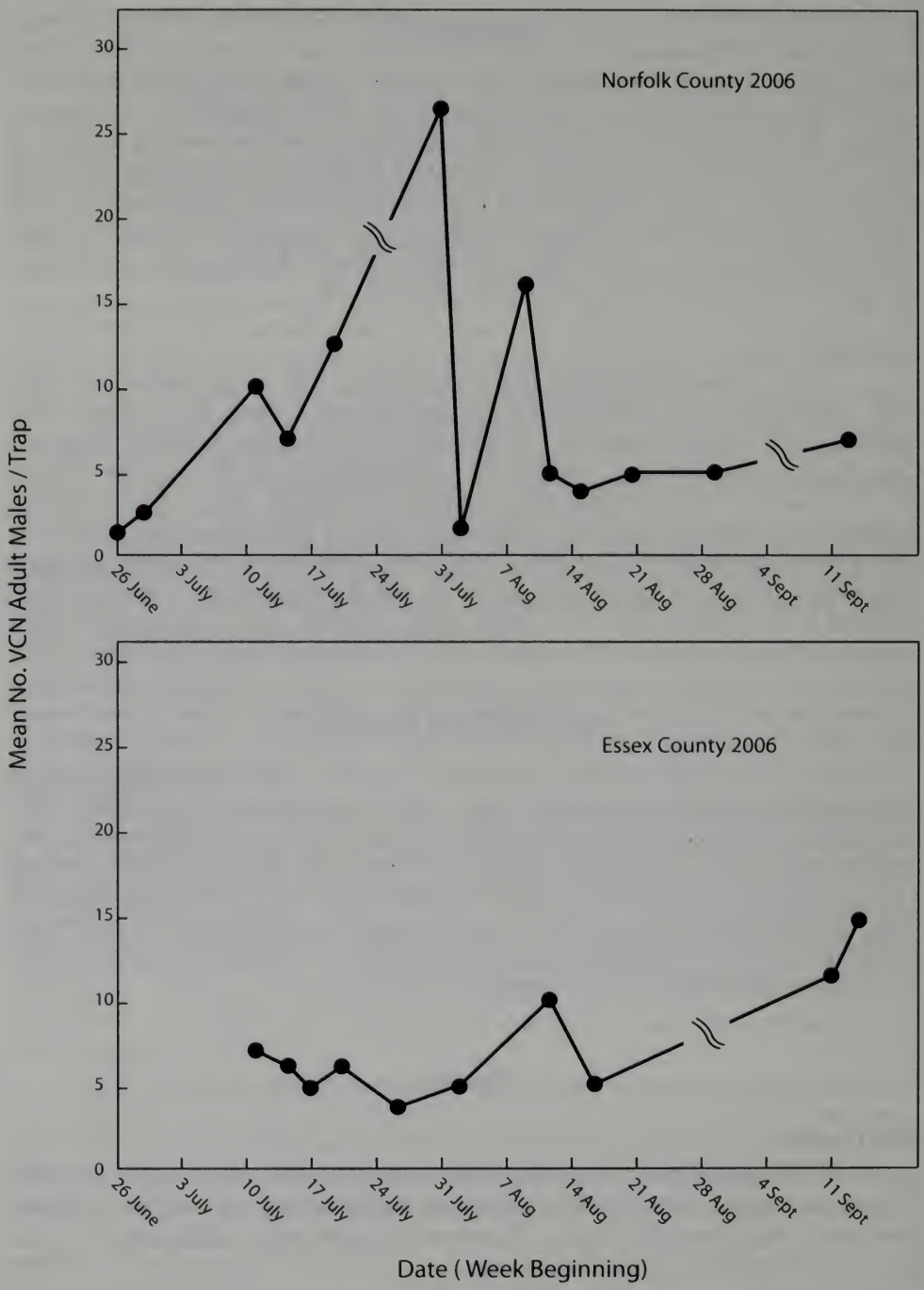

FIGURE 1. Mean numbers of adult male variegated cutworm (VCW) captured in pheromone traps in tomato fields in Norfolk and Essex Counties during Summer, 2006.

Insecticide Assays

Of the 4 insecticides commonly used for cutworm control, lambda-cyhalothrin was most toxic to 3rd-4th instar VCW > permethrin > chlorpyrifos > methomyl. Chlorantraniliprole was the most toxic of all the insecticides tested; spinosad and metaflumizone were the least toxic (Table 1). In tests on the direct contact toxicity of permethrin to different larval stages, at 0.001% solution, it was most toxic to 2nd instar (25% average corrected for mortality) > 3rd-4th (17%) > 5th (0%) larvae.

TABLE 1. Direct contact toxicity of 7 technical grade insecticides to 3rd-4th instar variegated cutworm larvae from a southwestern Ontario strain established from field populations collected in 2006.

Insecticide	Avg. corr. % mortality at % insecticide solution indicated				
	0.0001	0.001	0.01	0.1	1.0
Lambda-cyhalothrin	-	97	100	100	100
Permethrin	-	17	100	100	100
Chlorpyrifos	-	2	23	100	100
Methomyl	-	0	28	79	100
Chlorantraniliprole	15	100	-	-	-
Spinosad	-	2	14	78	100
Metaflumizone	-	0	3	66	84

TABLE 2. Comparison of the direct contact toxicity of technical grade methomyl, chlorpyrifos and permethrin to 3rd-4th instar variegated cutworm under identifical bioassay conditions, 1977 and 2007.

Insecticide	Year	Avg. corr. % mortality at % insecticide solution indicated			
		0.001	0.01	0.1	1.0
Methomyl	1977*	0	70	100	100
	2007	0	28	79	100
Chlorpyrifos	1977*	0	72	100	100
	2007	2	23	100	100
Permethrin	1977*	5	100	100	100
	2007	7	100	100	100

*Harris et al. 1977. Proc. Ent. Soc. Ont. 108: 63-68.

Discussion

In 2006, the July adult peaks noted in Norfolk County likely represented migratory adults, while that in August comprised the 1st generation arising from those adults (Fig. 1). McClanahan and Elliott (1976) reported that VCW had 3 peaks in Essex County in July, August and September suggesting that it had 2 generations in that area. Although adult collections were low at the Essex County sites in 2006, results were similar (Fig. 1). It appears that the warmer climatic conditions in Essex as compared to Norfolk County allow 2nd generation VCW to complete development.

The 2 pyrethroid insecticides, lambda-cyhalothrin and permethrin were the most toxic of the insecticides commonly used for cutworm control. Of the 3 reduced risk insecticides tested, chlorantraniliprole was at least as toxic as lambda-cyhalothrin (Table 1) suggesting that it might have a role in VCW IPM programs. Spinosad and metaflumizone were less toxic by direct contact, however it is known that both insecticides act primarily as stomach poisons. Results obtained with methomyl, chlorpyrifos and permethrin were compared with some obtained under identical bioassay conditions by Harris et al. (1977) (Table 2). VCW appears to have developed a relatively low level of resistance to methomyl, possibly due to exposure of the migrant population to use of that chemical on numerous host crops in North America. Results obtained with chlorpyrifos in the 2 years showed no indication of significant resistance development to that chemical.

While it has been suggested that unsatisfactory VCW control with permethrin on tomatoes could be due to resistance, results obtained with the 2006 Ontario strain were identical to those reported 30 years earlier (Harris et al., 1977). An explanation for less than acceptable control with permethrin can be found in past research which has shown that several factors influence the effectiveness of insecticides applied for VCW control including stage of larval development, climatic conditions and application method. Stage of larval development is very important. Similar to the results of this study, Harris et al. (1977) showed that, while 1st instar VCW were very susceptible, 3rd and 5th instar larvae were 2.9x and 3.7x more tolerant to permethrin, respectively. Harris and Kinoshita (1977) demonstrated that, like DDT, pyrethroid toxicity can be negatively correlated with temperature – permethrin was 7.4x more toxic to 3rd instar VCW at 15° as compared to 32°C. Finally application method has a major role. For example, VCW spends the larval stage on, or very close to, the soil surface hidden under dense foliage. While soil surface applications with permethrin were effective, foliar applications were more so (Harris and Svec 1968; Harris et al. 1977).

Results of this study show that the pyrethroid insecticides remain highly toxic to the Ontario VCW populations found in southern Ontario and that there is no evidence of resistance to permethrin. Lack of insecticide effectiveness in the field is undoubtedly due to limited mobility of the cutworms once they become established under the plant canopy. To achieve adequate control, insecticides should be applied when early instar larvae are present, under moderate to cool climatic conditions in a manner resulting in the most effective penetration of the plant canopy as possible.

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ADDITIONS TO THE SPIDER FAUNA OF QUÉBEC (ARANEAE)

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Abstract *J. ent. Soc. Ont.* 139: 27–39

The knowledge of the spider fauna of Québec, Canada, is updated with provincial records gathered in bio-inventories and ecological projects. Eight species are reported for the first time in the province: Araneidae: *Araneus guttulatus* (Walckenaer, 1841), *Eustala cepina* (Walckenaer, 1841), *Mastophora hutchinsoni* Gertsch, 1955; Linyphiidae: *Disembolus sacerdotalis* (Crosby and Bishop, 1933); Salticidae: *Admestina wheeleri* Peckham and Peckham, 1888; Tetragnathidae: *Leucauge venusta* (Walckenaer, 1841); Theridiidae: *Dipoena appalachia* Levi, 1953, and *Keijia alabamensis* (Gertsch and Archer, 1942). Three of these species are found in Canada for the first time. Collecting data, overview of distribution, and diagnoses are provided for each species, in addition to detailed illustrations to facilitate identification. These records bring the total to 667 known spider species in the province (31 families), leaving 129 species as probable records, and an estimated 45 'unpredictable' species records, for a projected total of 841 species.

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Introduction

The knowledge of the spider fauna of Québec has benefited from a solid foundation established with species lists (Bélanger and Hutchinson 1992; Hutchinson and Bélanger 1994; Paquin et al. 2001) and a monograph for species level identification (Paquin and Dupérré 2003). Since, a few papers clarified taxonomic problems (Dupérré and Paquin 2005), described unknown sexes (Pickavance 2004; Dupérré et al. 2006), described new taxa (Dupérré and Paquin 2007a, b; Lopardo et al. 2008) and reported new records of species

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found in the province (Paquin and Dupérré 2006). Paquin et al. (2008a) also documented the occurrence of three species that maintain viable populations in an artificial ecosystem of Montréal, but are not yet established on the territory.

In this paper, we propose a second update of the faunistic knowledge for Québec spiders – following Paquin and Dupérré (2006) – with eight new species records for the province. We provide accurate illustrations with angles and style comparable to Paquin and Dupérré (2003) to ease comparison and species level identification. Up-to-date faunistic knowledge facilitates the detection of introductions, the recognition of invasive species, or potential changes in species distribution. Shifts in ranges may be induced by climatic changes (Dukes and Mooney 1999; Parmesan and Yohe 2003) that push further north species that were earlier restricted to regions south of the province.

Materials and Methods

Specimen Collection

The records reported here were gathered from three bio-inventories/ecological projects carried out in different regions of Québec: the spiders of the Parc National de la Yamaska (Paquin et al. 2008b); the arboreal spider fauna of sugar maple forests in southern Québec: Oka and Mont-Saint-Bruno National Parks, and the Mont Saint-Hilaire world biosphere reserve (M. Larrivée); and the spider bio-inventory of the Réserve Nationale de faune du cap Tourmente (C. Simard).

Specimen Examination

Specimens were examined in 70% ethanol under an SMZ-U Nikon dissection microscope. A Nikon Coolpix 950 digital camera attached to the microscope was used to photograph all the structures to illustrate. The digital photos were used to trace proportions and the illustrations were detailed and shaded by referring back to the structure under the microscope. Female genitalia were excised using a sharp entomological needle and transferred to lactic acid to clear non-chitinous tissues. A temporary lactic acid mount was used to examine the genitalia under the compound microscope. After each record, an acronym indicates where the specimens are deposited: CPAD: Collection of Paquin-Dupérré (Shefford, Québec, Canada); CNC: Canadian National Collection of Insects and Arachnids (Ottawa, Ontario, Canada).

The general terminology follows Ubick et al. (2005), but Dondale et al. (2003), Levi (1953, 1957), Millidge (1981) and Piel (1992) were also consulted. Latitude and longitude data are given in decimals and should be considered an approximation. Other acronyms used are MCZ (Museum of Comparative Zoology at Harvard, Cambridge, Massachusetts, U.S.A.), and AMNH (American Museum of Natural History, New York, New York, U.S.A.). *Abbreviations used:* A atrium, C conductor, CD copulatory ducts, CO copulatory openings, E embolus, M suprategular apophysis, membranous part, MA median apophysis, PTA palpal tibia apophysis, R radix, S spermathecae, SD sperm duct, SubTA subterminal apophysis, TA terminal apophysis, TP tailpiece.

Family Araneidae

***Araneus guttulatus* (Walckenaer, 1841)**

(Figs. 1–5)

Material Examined. Canada: Québec: Parc National de la Yamaska [45.42°N, 72.39°W] 1♀ 12.ix.2006, Berlese extraction of litter, deciduous forest in regeneration, A. Mochon (CPAD); 1♀ 18.–25.vii.2006, water pan, mixed forest, A. Mochon (CPAD); Parc National d'Oka [45.49°N, 74.01°W] 1♀ 10.viii.05, beating, sugar maple maple (*Acer saccharum* Marsh.) canopy, M. Larrivée (CNC); Parc National du Mont-Saint-Bruno [45.55°N, 73.32°W] 1♂ 29.vi.2006, 1♀ 07.vi.2006, beating, sugar maple canopy, M. Larrivée (CNC); 1♀ 24.viii.2006, beating, American beech (*Fagus grandifolia* Ehrh.) canopy, M. Larrivée (CNC); 1♂ 29.vi.2006, beating, sugar maple understory, M. Larrivée (CNC).

Diagnosis. *Araneus guttulatus* bears resemblance to *Araneus juniperi* (Emerton, 1884) and *Araneus cingulatus* (Walckenaer, 1841). Both males and females are distinguished by their abdominal pattern and central black mark (Figs. 1, 3). Males are characterized by their stout, distally hooked embolus (E) (Fig. 2). Females are differentiated by the large copulatory openings (CO) of the epigynum (Fig. 4) and close together and ovoid spermathecae (S) (Fig. 5).

Distribution. U.S.A.: southern Georgia to Arkansas, Maine to Wisconsin (Dondale et al. 2003). Canada: Québec and Ontario.

Remarks. The abdominal pattern of *A. guttulatus* (Figs. 1, 3) is quite different from *A. juniperi* which is bright green with pale longitudinal bands (see Paquin and Dupérré 2003, Fig. 276). However, coloration tends to fade rapidly with preserved specimens; color characters may guide identification of fresh specimens, but are not reliable for older ones.

***Eustala cepina* (Walckenaer, 1841)**

(Figs. 6–8)

Material Examined. Canada: Québec: Parc National de la Yamaska [45.42°N, 72.39°W] 1♂ 20.–27.vii.2006, water pan, deciduous forest in regeneration, A. Mochon (CPAD); 1♂ 1♀ 04.vii.2000, beating, C. Chantal (CPAD); Lajemmerais, Varennes [45.68°N, 73.43°W] 1♀ 23.vii.2000, beating, C. Chantal (CPAD); Pontiac, Les Collines-de-l'Outaouais, Quyon [45.51°N, 76.23°W] 1♀ 24.vii.1992, sand pit, sweeping, L. LeSage (CPAD); Gatineau Park, near Eardley [45.51°N, 76.23°W] 1♂ 14.vi.2001, deciduous woods, D.J. Buckle (CPAD).

Diagnosis. *Eustala cepina* most resembles *Eustala anastera* (Walckenaer, 1841) (see Dondale et al. 2003, Figs. 600–611; Paquin and Dupérré 2003, Figs. 309–310), but *E. cepina* is somewhat smaller in size. Males can be distinguished by their shorter terminal apophysis (TA) not extending beyond the subterminal apophysis (SubTA) (Fig. 6). Females can be differentiated by the smaller spermathecae (S) and slender, sinuous copulatory ducts (CD) not folded on themselves (Fig. 8).

Distribution. Eastern North America (see Dondale et al. 2003, map 72), reaching its northern limit in southern Canada: Nova Scotia, Québec and Ontario.

***Mastophora hutchinsoni* Gertsch, 1955**
(Figs. 9–13)

Material Examined. Canada: Québec: Mont-Saint-Hilaire [45.54°N, 73.19°W] 1 juvenile, 25.viii.2006, beating, American beech canopy, M. Larrivée (CNC).

Diagnosis. Both males and females of *Mastophora hutchinsoni* are easily distinguished from all other Araneidae found in Canada by the forked tubercles on the carapace (Figs. 9, 10) and the shape of their abdomen (Figs. 9, 10).

Distribution. U.S.A.: from Minnesota to New Hampshire, south to South Carolina (see Dondale et al. 2003: map 29) and Canada: Québec.

Remarks. The highly distinctive feature of the tubercles on the carapace leaves no doubt about the specimen identification, despite its juvenile stage. The species was included in Dondale et al. (2003) because its presence was suspected in Canada, particularly in Southern Ontario. This record represents an important range extension.

Family Linyphiidae
***Disembolus sacerdotalis* (Crosby and Bishop, 1933)**
(Figs. 14–18)

Material Examined. HOLOTYPE: *Cochlembolus sacerdotalis* Crosby and Bishop, 1933, male (AMNH). Label 1: 'Karners, N.Y. Mar.24, 1923'. Label 2: '*Disembolus sacerdotalis* (Crosby and Bishop), Millidge 1979'.

New Material. Canada: Québec: Réserve Nationale de faune du cap Tourmente [47.08°N, 70.78°W] 1♂ 27.xi.2004, sifting, maple forest, C. Simard (CPAD).

Diagnosis. *Disembolus sacerdotalis* most resembles *Disembolus anguineus* Millidge, 1981, and *Disembolus convolutus* Millidge, 1981 in the shapes of the carapace, cephalic lobe, pits and sulci (Fig. 14). *Disembolus sacerdotalis* can be distinguished by the large loose loop of the embolus (E) (Figs. 15, 17), larger suprategular apophysis membranous part (M) (Fig. 15), the longer and narrower end of tailpiece (TP) (Fig. 15) and the form of the palpal tibial apophysis (PTA) (Fig. 18).

Distribution. Northeastern North America: New York (Buckle et al. 2001), Massachusetts and Québec.

Remarks. This first Canadian record is only the third known specimen, including the holotype examined to confirm our identification. The other known specimen is curated at the Museum of Comparative Zoology (MCZ #60781, Buzzards Bay, Massachusetts, iv.1966,

L. Pinter). The palp of the holotype illustrated in Millidge (1981) and Draney and Buckle (2005) is slightly expanded when compared with the specimen illustrated here. Because of the rarity of the species, its occurrence in Québec was not suspected by Hutchinson and Bélanger (1994). The female of the species is still unknown.

Family Salticidae
***Admestina wheeleri* Peckham and Peckham, 1888**
(Figs. 19–24)

Material Examined. Canada: Québec: Parc National de la Yamaska [45.42°N, 72.39°W] 1♀ 05.ix.2006, beating, deciduous forest in regeneration, A. Mochon (CPAD); 1♂ 10.x.2006, beating, mixed forest, A. Mochon (CPAD).

Diagnosis. *Admestina wheeleri* most resembles *Admestina tibialis* (C.L. Koch, 1846) (see Figs. 2151–2153 in Paquin and Dupérré 2003). Males are differentiated by the narrower and longer embolus (E) (Fig. 21), females by the smaller copulatory openings (CO) (Fig. 22) separated by a distance of four times their diameter from the genital groove (see Piel 1992), and the longer and more sinuous copulatory ducts (CD) (Fig. 23).

Distribution. Northern United States: Maine to North Dakota; Canada: Québec to Manitoba.

Remarks. The species was not included in the list of probable records of Hutchinson and Bélanger (1994), but based on the distribution given by Piel (1992), its presence in Québec was highly probable.

Family Tetragnathidae
***Leucauge venusta* (Walckenaer, 1841)**
(Figs. 25–29)

Material Examined. Canada: Québec: Parc de la Gatineau, Lusk trail [45.56°N, 75.95°W] 1♀ 01.viii.2001, M. Larrivée (CNC).

Diagnosis. Both males and females of *Leucauge venusta* are easily distinguished from all other Canadian Tetragnathidae by the presence of a cluster of trichobothria on the prolateral surface of femur IV (Fig. 25) and the robust form and coloration of the abdomen (Fig. 27) (see also Dondale et al. 2003).

Distribution. Eastern North America with a few records from Southwestern United States (see Dondale et al. 2003, map 5); Canada: Ontario and Québec.

Remarks. The species was not included in the list of probable records of Hutchinson and Bélanger (1994), but based on the distribution given by Dondale et al. (2003), its presence in Québec was predictable.

Family Theridiidae
***Dipoena appalachia* Levi, 1953**
(Figs. 30–31)

Material Examined. Canada: Québec: Parc National de la Yamaska [45.42°N, 72.62°W] 1♂ 04.vii.2006, beating, mixed forest, A. Mochon (CPAD); 1♂ 04.–11.vii.2006, water pan, mixed forest, A. Mochon (CPAD); 1♂ 27.vi.–04.vii.2006, pitfall, mixed forest, A. Mochon (CPAD).

Diagnosis. *Dipoena appalachia* closely resembles *Dipoena nigra* (Emerton, 1882) but differs by the evenly yellow-brown legs lacking the much darker femur and patella I present in *D. nigra.* The male palp is characterized by the sperm duct (SD) acutely curved (Fig. 31), the pointed distal part of the radix (R) and the conductor (C) form (Fig. 31).

Distribution. Southeastern United States to Maryland (Levi 1953); Canada: Québec.

Remarks. *Dipoena appalachia* displays variability in the palp configuration (see Levi 1953, Figs. 24, 25, 27). However, the specimens collected in the Parc National de la Yamaska showed no variation. These first Canadian records represent an unexpected range extension for the species. The female of *D. appalachia* is unknown.

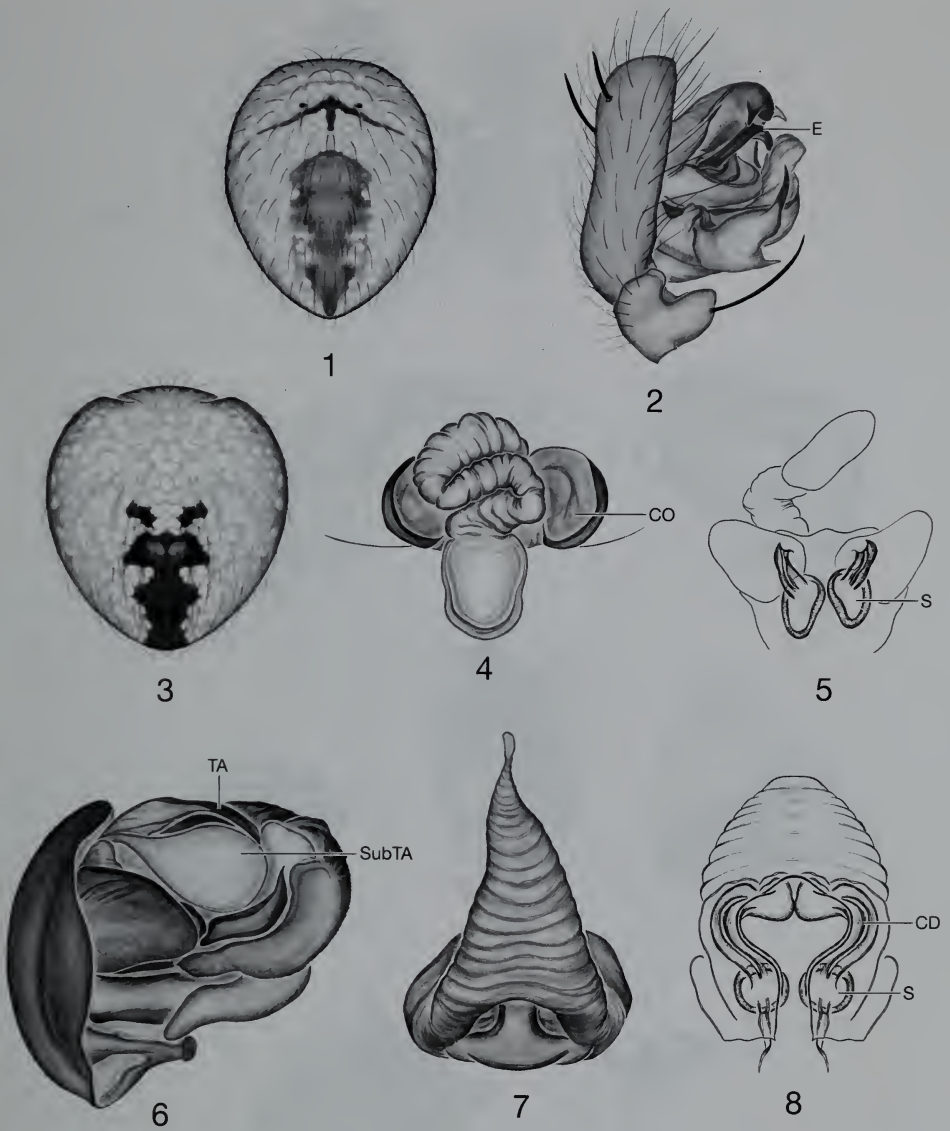
***Keijia alabamensis* (Gertsch and Archer, 1942)**
(Figs. 32–36)

Material Examined. Canada: Québec: Parc National de la Yamaska [45.44°N, 72.60°W] 1♀ 27.vi.2006, beating, open field, A. Mochon (CPAD); 1♀ 27.vi.–04.vii.2006, water pan, sugar maple forest, A. Mochon (CPAD); 1♀ 29.viii.2006, beating, sugar maple forest, A. Mochon (CPAD); Parc National du Mont-Saint-Bruno [45.55°N, 73.32°W] 1♂ 15.v.2006, 1♀ 21.vi.2006, beating, sugar maple understory, M. Larrivée (CNC); Parc National d'Oka [45.49°N, 74.02°W] 1♀ 25.v.2006, beating, sugar maple understory, M. Larrivée (CNC).

Diagnosis. *Keijia alabamensis* is distinguished by the combination of the following characters: male palp with thick embolus (E) (Fig. 32), long and pointed median apophysis (MA) (Fig. 32). Females are characterized by their dark abdomen with a distinct apical white mark (Fig. 33), epigynum with large, oval atrium (A) (Fig. 34), copulatory ducts (CD) large, touching medially and narrowing down into one loop (Figs. 35, 36). The genitalic configuration of the species is quite unique, especially the rounded shape of the atrium and the male embolus, which allows an easy distinction from its congeneric members.

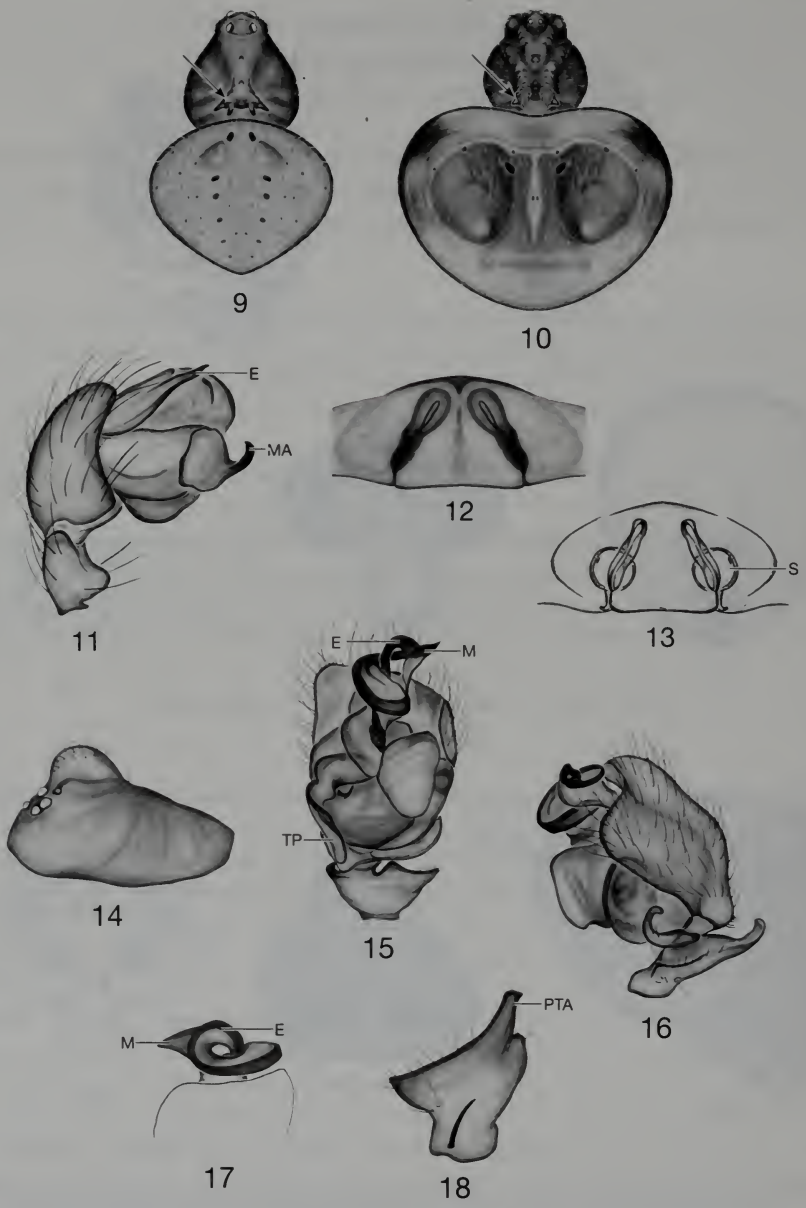
Distribution. Eastern North America, north to Québec and New Brunswick, with a few records from California (Levi 1957).

Remarks. *Keijia alabamensis* has been transferred from *Theridion* by Yoshida (2001), with three other North American species (Platnick 2007). The species was included on the list of probable records for Québec of Hutchinson and Bélanger (1994).



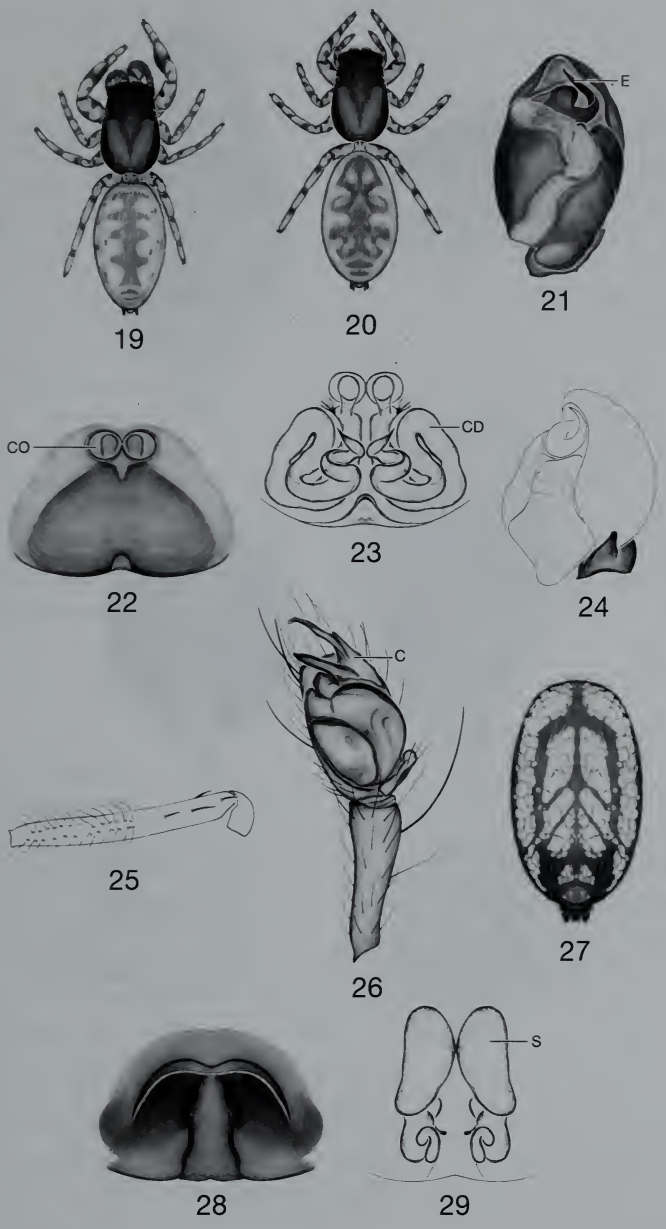
FIGURES 1–5. *Araneus guttulatus* 1, male abdomen, dorsal view; 2, palpus, ventral view; 3, female abdomen, dorsal view; 4, epigynum, ventral view; 5, spermathecae, posterior view.

FIGURES 6–8. *Eustala cepina* 6, palpus, prolateral view; 7, epigynum, ventral view; 8, spermathecae, posterior view.



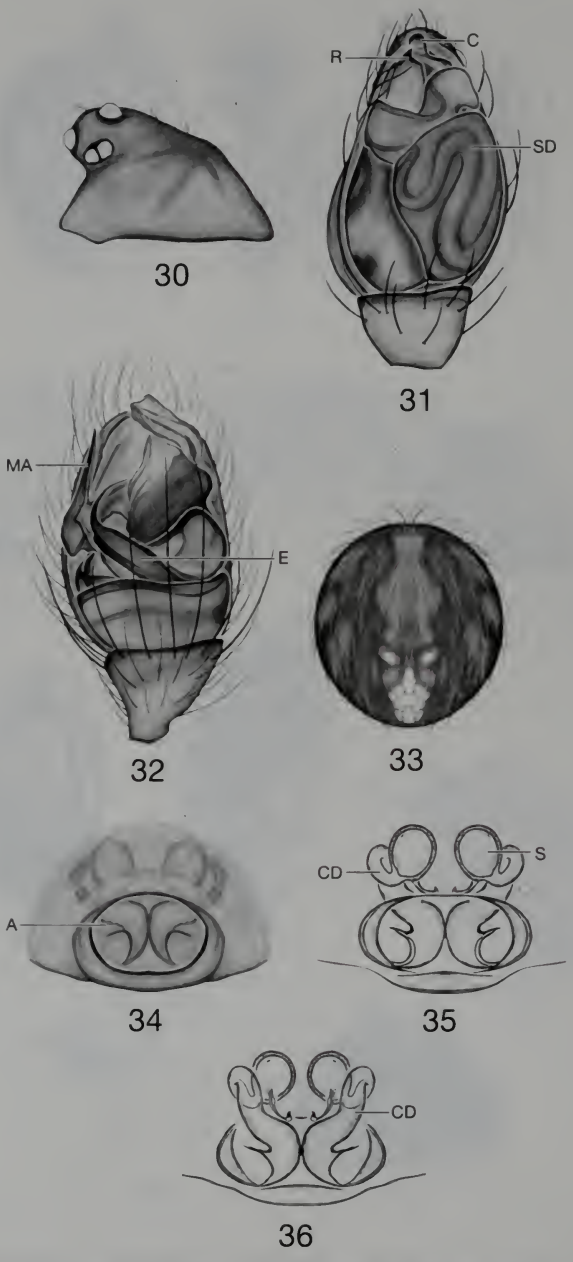
FIGURES 9–13. *Mastophora hutchinsoni* 9, male, dorsal view, arrow points to tubercles; 10, female, dorsal view, arrow points to tubercles; 11, palpus, prolateral view; 12, epigynum, ventral view; 13, spermathecae, ventral view.

FIGURES 14–18. *Disembolus sacerdotalis* 14, male carapace, lateral view; 15, palpus, ventral view; 16, palpus, retrolateral view; 17, tip of palpus, dorsal view; 18, palpal tibia, dorsal view.



FIGURES 19–24. *Admestina wheeleri* 19, male, dorsal view; 20, female, dorsal view; 21, palpus, ventral view; 22, epigynum, ventral view; 23, spermathecae, dorsal view; 24, palpus, retrolateral view.

FIGURES 25–29. *Leucauge venusta* 25, femur IV, prolateral surface; 26, palpus, ventral view; 27, female abdomen, dorsal view; 28, epigynum, ventral view; 29, spermathecae, ventral view.



FIGURES 30–31. *Dipoena appalachia* 30, male carapace, lateral view; 31, palpus, ventral view.

FIGURES 32–36. *Keijia alabamensis* 32, palpus, ventral view; 33, female abdomen, dorsal view; 34, epigynum, ventral view; 35 spermathecae, ventral view; 36, spermathecae, dorsal view.

Discussion

In the first update of the spiders of Québec, Paquin and Dupérré (2006) estimated that 35% of the records reported were unpredictable, because species ranges were too far from Québec, or these species were too rare to allow any sound predictions. From the eight records reported in the present paper, three are considered unpredictable, confirming the important proportion of such occurrences (37.5%) in faunistic updates.

Pickavance and Dondale (2005) presented a comparative table of spider richness per province, in which Québec was represented by 617 species and 28 families. The present update brings the total to 667 species and 31 families. This leads to a re-evaluation of the predicted spider fauna for the province: 667 known species (present paper); 129 probable species (updated from Paquin et al. 2001); 45 unpredictable species records (35% of probable records), suggesting a potential of 841 species for Québec. The parameter not included in this equation remains the number of species that are still undescribed. There is no doubt that many species (described or not) await discovery and that future bio-inventories and ecological projects will provide additional records and species. This is particularly true for habitats, microhabitats, and regions that are not well understood such as bogs, fens, forest canopy, high altitude habitats and northern localities.

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SUSCEPTIBILITY OF TWO STRAINS OF AMERICAN SERPENTINE LEAFMINER (*LIRIOMYZA TRIFOLII* (BURGESS)) TO REGISTERED AND REDUCED RISK INSECTICIDES IN ONTARIO

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Abstract

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American serpentine leafminer, *Liriomyza trifolii* (Burgess), is a pest of floriculture greenhouses in Ontario. Growers rely on chemicals to provide acceptable pest control and, consequently, this leafminer has developed resistance to many insecticides. Our objectives were to determine if the American serpentine leafminer in Ontario has developed resistance to registered insecticides and to evaluate effectiveness of reduced risk insecticides with potential for inclusion in management programs.

Two leafminer cultures were established – one collected from greenhouses in the Niagara region, the other being an insecticide susceptible strain never exposed to any of the test insecticides. Dosage-mortality curves were constructed using a leaf dip bioassay in which newly infested bean leaves were dipped in formulated insecticide solutions and larvae were allowed to develop until adults emerged. At the LC_{50}, the Ontario strain was resistant to abamectin (17.5x) and cyromazine (10.2x) and showed low levels of resistance to spinosad (2.8x) and chlorantraniliprole (3.0x) – such low resistance levels also could be due to natural variation in the strains. A comparison of LC_{95} to application rates showed that the amount of insecticide required to kill 95% of the Ontario strain would be much higher than the recommended rate for cyromazine, just within the rate for abamectin, and lower that suggested rates for spinosad and chlorantraniliprole. While the LC_{95}s for spinosad and chlorantraniliprole were lower than suggested application rates. Nevertheless, the low level resistance shown by the Ontario strain suggests that these 2 insecticides may have the potential to develop higher levels of resistance over time.

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Introduction

In 2006, Ontario produced about 14 million potted and 21 million cut chrysanthemums (*Dendranthema grandiflora* Tzvelev), and 3 million potted and 30 million cut gerberas (*Gerbera jamesonii* H. Bolus ex J. D. Hook) (Statistics Canada 2006). The American serpentine leafminer (*Liriomyza trifolii* (Burgess)) attacks both crops causing aesthetic damage to leaves when larvae feed on leaf mesophyll producing serpentine mines. It was reported to be a major pest in Ontario floriculture greenhouses in the late 1970s (Broadbent 1982) but has been kept under control by insecticides. Floriculture producers often rely on heavy application of chemicals to control pests because consumers demand a high quality product free from aesthetic damage (Jones et al. 1986; Parrella 1987; Gullino and Wardlow 1990). There are only a few insecticides registered for American serpentine leafminer control in Canada (Chaput 2000). Abamectin and cyromazine are registered systemic insecticides acting on leafminer larvae. Permethrin can be used against leafminer adults but resistance to permethrin may be present in the population (Keil and Parrella 1990; Murphy 2004). Unfortunately, the short generation time and high reproductive rate of this leafminer combined with excessive insecticide use have caused it to develop resistance to many insecticides (Jones et al. 1986; Parrella 1987; Keil and Parrella 1990). In 2004, growers in Ontario reported difficulty in controlling it with registered insecticides (G. D. Murphy, pers. comm., 2005), suggesting that it was becoming resistant to both cyromazine and abamectin. In a survey of Ontario chrysanthemum and gerbera growers Conroy et al. (2007) found that 22% of respondents had observed failure to effectively control the leafminer with insecticides. Reduced risk insecticides, such as spinosad and chlorantraniliprole, need to be tested for control of this leafminer and compatibility with integrated pest management programs.

The objectives of this study were to determine if the American serpentine leafminer has developed resistance to currently registered insecticides in Ontario since control failures have been observed and to evaluate the effectiveness of reduced risk insecticides with potential for inclusion in management programs.

Materials and Methods

American Serpentine Leafminer Culture

Two American serpentine leafminer colonies were established. A reference strain was obtained from Dr. Scott Ferguson (Syngenta Crop Protection, Florida). This strain had been reared in the laboratory for over 20 years and had never been exposed to insecticides currently registered for leafminer control. The second strain was collected from chrysanthemum and gerbera greenhouses in the Niagara region of Ontario in 2006-2007. Resistance may have developed as a result of the history of insecticide use in affected greenhouses, or more likely given the synchronicity of problems in a number of greenhouses, it was imported along with plant material from the United States where resistance to currently registered insecticides has been documented (Ferguson 2004; G. D. Murphy, pers. comm., 2005). Both strains were kept in plexi-glass cages in environmental chambers at 26 ± 2°C, 50-70% RH, and 18:6 L:D. The strains were kept in separate buildings following

proper quarantine protocols. Insects were reared on green beans (cv. Provider®, Veseys Inc.) grown in ProMix®, and held in growth chambers at 25 ± 2°C and 18:6 L:D. A 10 cm filter paper covered in dilute honey was supplied for carbohydrate. Greenhouse collected leafminers were added to the Ontario strain culture every 1-2 months to maintain similar genetic diversity to field populations.

Leaf Dip Bioassay

Bioassay methods were modified from Ferguson (2004). Four to 6 bean plants at the 2-4 leaf stage were exposed to leafminer colonies for 1-3 h depending on colony fitness (number of adults). Plants were removed from the colony and placed in environmental chambers (26 ± 5°C, 30-50% RH, and 18:6 L:D) for 2-3 days or until small mines appeared on the leaves. Mines on each leaf were counted and marked with a permanent marker. Leaves were removed and placed in flower picks filled with deionized water on a metal drying rack on a cafeteria tray lined with paper towel. Seven to15 mines per leaf were used per replication for both strains. The number of mines per leaf was difficult to control due to the varying number of adults in each colony for both strains at any given time. A higher number of mines (> 20/leaf) decreased overall survivorship.

Cyromazine (Citation® 75 WP, Syngenta Crop Protection Canada), abamectin (Agri-Mek®/Avid® 1.9 % EC, Syngenta Crop Protection Canada), spinosad (Success® 480 SC, Dow AgroSciences) and chlorantraniliprole (Altacor™ 35% WG, DuPont Canada) were tested. *Note: Altacor™ 35 WG (DuPont Canada) was the only formulation available at the onset of this project. Coragen™ 20 SC (DuPont Canada) is now the formulation appropriate for greenhouse use.* The formulated insecticides were mixed with deionized water. Super Spreader® (United Agri Products Canada Inc.) wetting agent (0.05 ml) was added to each concentration to enhance wetting of the leaves (Ferguson 2004). Deionized water mixed with 0.05 ml wetting agent was used as the control. One or 2 infested leaves with a total of at least 7 mines were dipped into a beaker containing 200 ml of the insecticide solution for 5 s and then placed on a rack in a fumehood to dry for 1 h. Leaves were then removed from the flower picks and placed in 10 cm plastic Petri® dishes lined with filter paper. Petri dishes were sealed with parafilm. Post-treatment containers were kept in an environmental chamber at 26 ± 5°C, 30-50% RH, and 18:6 L:D for 7 days until pupation. Pupae were then counted, the leaves were removed and the pupae were returned to the Petri dishes in the chamber for 2-4 weeks until all adults had eclosed and died. Emerged adults were then counted. Preliminary screening tests were done to determine a range of concentrations (15-95% mortality) appropriate for construction of a dosage mortality curve. Tests were replicated 4 times.

Data Analysis

Abbott's formula (Abbott 1925) was used to correct for control mortality (< 15%). Data were analyzed using SAS version 9.1 (SAS institute, Cary, NC) with a type 1 error rate of $\alpha = 0.05$. The probit procedure was used to determine LC_{50} and LC_{95} values by log transforming the data to fit the probit scale. A Chi-square goodness of fit test was used to test the significance of the probit regression and determine the fiducial limits. The difference between the 2 LC_{50} values was deemed significant if there was no overlap of the 95% fiducial limits. Resistance ratios were calculated by dividing the resistant Ontario

strain LC_{50} by the susceptible strain LC_{50}.

Results and Discussion

Cyromazine, abamectin and spinosad resistance have been documented in Florida (Ferguson 2004) but only anecdotal evidence has been reported from Ontario since 2004 (G. D. Murphy, pers. comm., 2005). Defining "resistance", particularly at low levels can be arbitrary. Comparison of fiducial limits, as suggested by Robertson et al. (2007) indicates, that as the limits of the LD_{50} of the insecticide susceptible and Ontario leafminer strains did not overlap, the latter was resistant to abamectin (17.5x) and cyromazine (10.2x) and also showed low levels of resistance to spinosad (2.8x) and chlorantraniliprole (3.0x) (Table 1) - these low levels of "resistance" also could be due to natural variation in the Ontario population (Robertson et al. 2007) or to enhanced susceptibility of the reference strain reared for many years under controlled laboratory conditions (ffrench-Constant and Rousch 1990).

Resistance also can be defined as failure of an insecticide to control an insect pest in the field (Ball 1981). The survey of the Ontario chrysanthemum and gerbera growers discovered that 22% of respondents observed failure to control American serpentine leafminer with registered insecticides (Conroy et al. 2007). Comparing the LC_{95} to the recommended field rates (Table 2) shows that the amount of insecticide needed to kill 95% of the Ontario strain would be much higher (4-5x) than that recommended for cyromazine. The recommended rate for abamectin was close to the LC_{95} suggesting that resistance in Ontario greenhouses may not be present. However, since the population was not gathered from a single source it is possible that resistance varies between greenhouses and could be higher in some, causing control failures. A low level of insecticide resistance was observed with spinosad (Table 1). It is registered in the United States for leafminer control and thus it is not surprising that the Ontario strain, possibly imported from the United States on infested propagation material would show decreased susceptibility to this insecticide. However, the recommended application rate would appear to be adequate to provide effective leafminer control (Table 2).

Chlorantraniliprole is a new insecticide with a unique mode of action. Comparing the application rate to the LC_{95} suggests that the amount needed to provide 95% control also is lower than the suggested application rate (Table 2). Nevertheless, the low level of insecticide resistance shown by the Ontario strain to this product suggests that it may have the potential to develop a higher level of resistance (Table 1). If chlorantraniliprole or spinosad is registered for American serpentine leafminer control, it should be in the context of an integrated pest management program minimizing use in order to delay resistance development.

Conclusions

These results stress the importance of developing a multifaceted integrated pest management program for American serpentine leafminer control in Ontario. Insecticides alone will not control it without rapid development of insecticide resistance. An IPM

TABLE I. LC_{50} and level of resistance of 2 American serpentine leafminer strains (Ontario = O; Susceptible = S) exposed to formulated insecticides, either registered (abamectin, cyromazine) or reduced risk (chlorantraniliprole, spinosad), using a leaf dip bioassay.

Insecticide	Strain	N	Slope	Pearson $\chi 2$	LC_{50}[1]	95 % Fiducial Limits	Resistance Ratio O/S
Abamectin	O	249	1.52	0.262	1.05	0.72 - 1.40	17.5
	S	318	1.94	0.219	0.06	0.05 - 0.07	
Cyromazine	O	343	1.40	0.453	33.6	26.0 - 44.0	10.2
	S	220	3.13	0.087	3.28	2.24 - 4.41	
Chlorantraniliprole	O	289	1.99	0.292	0.63	0.53 – 0.76	3.0
	S	295	3.10	0.169	0.21	0.19 – 0.24	
Spinosad	O	192	2.14	0.673	2.82	2.01 - 3.67	2.8
	S	177	2.31	0.121	1.00	0.81 - 1.28	

[1] Concentrations expressed as ppm.

TABLE 2. LC_{95} for the Ontario American serpentine leafminer strain exposed to formulated insecticides using a leaf dip bioassay compared to recommended application rates.

Insecticide	LC_{95}[1]	95 % Fiducial Limits	Recommended Application Rates[1]
Cyromazine	506	301 – 1051	141
Abamectin	12.7	7.9 – 27.8	11
Spinosad	16.5	12.0 – 26.5	24
Chlorantraniliprole	4.3	2.6 – 11.0	9

[1] Concentrations and rates are expressed as ppm.

program that decreases the number of insecticide applications, rotates products with different modes of action and incorporates biological control would be more effective.

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ALTERNATIVE mRNA SPLICE VARIANTS IN *DROSOPHILA* DL2 CELLS FOLLOWING FLOCK HOUSE VIRUS INFECTION

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Abstract *J. ent. Soc. Ont.* 139: 49–58

There is a paucity of information regarding the responses of the insect immune system to viral infection. In other metazoan cells and tissues mRNA splicing variants are frequently associated with viral infection. Here we analyzed transcripts of the DNA repair gene 8-oxoguanine DNA glycosylase generated prior to, and post, infection of *Drosophila melanogaster* macrophage-like DL-2 cells by Flock House Virus (FHV). In mock-infected controls we observed that 2% of the transcripts were incompletely processed, maintaining some but not all introns. Eight hours post-FHV infection of the DL-2 cells, we observed a seven fold increase in the frequency of immature *Ogg1* transcripts. Moreover, there was a change in the introns retained in the transcripts observed compared with mock-infected controls, including completely unspliced transcripts. Surprisingly, the frequency of immature transcripts was reduced to control levels by 12 hours post-inoculation and remained relatively low up to 48 hrs. These results support the conclusion that viral infection may be accompanied by a host-mediated partial inhibition of mRNA splicing factors. This phenomenon has the potential to generate novel splice variants that are neither directly useful to the host nor the infecting virus but have the potential to degrade the transmission of genetic information. To our knowledge this the first report that viral infection may elicit general splicing instability in insects.

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Introduction

Multicellular host organisms have evolved under constant threat of infection from viruses, and consequently they employ numerous anti-infection defenses, such as inflammation, apoptosis, RNA interference, and innate and adaptive immune responses (Tschopp et al. 1998; Dostert et al. 2005; Zambon et al. 2006). At the same time viruses have evolved various strategies to evade host defenses and usurp the cellular machinery for their own reproductive requirements (Li et al. 2002). Understanding the interactions of viruses with their insect hosts is particularly important because insect viruses can cause

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severe problems, both for insects (de Miranda et al. 2008) and for humans, as for example in the case of tick-borne encephalitis virus or the mosquito-born West Nile virus (Brauchli et al. 2008; Orshan et al. 2008). Moreover, elucidation of the interactions between insect viruses and their hosts would help in increasing the efficacy of the many schemes currently underway to use viruses as agents of biological control against insects and for high-throughput production of proteins in insect hosts (Nicholson 2007; Possee et al. 2008).

In order to elucidate host-virus interactions in insects, an appropriate model system is *Drosophila* because it has been the subject of extensive genetic investigations and its gene makeup is largely known (Warren et al. 2006; Lai et al. 2007). *Drosophila* relies on a large repertoire of innate immune defense mechanisms against microbial infections (Lemaitre and Hoffman, 2007). However, with respect to viral infections, RNAi is the only known effector mechanism that has been identified in *Drosophila* (Wang et al. 2006). Microarray analysis of genes expressed in *Drosophila* infected with *Drosophila* C virus (DCV) indicated a transcriptional response to viral infection that differed from bacterial or fungal infection (Dostert et al. 2005). In particular, viral infection was accompanied by an upregulation of the JAK-STAT pathway, which also plays an important role in mammalian viral infections. Several prominent immune reactions and pathways are shared between *Drosophila* and mammals, such as phagocytosis and the Toll-mediated pathways (Lemaitre and Hoffman, 2007). These similarities point to a common evolutionary ancestry in immune reactions to microbial infection and suggest other common reactions. In mammals, viral infection may also be accompanied by changes in host post-transcriptional RNA processing and translation (Adair et al. 2006). Some mammalian DNA and RNA viruses, lacking introns, such as *Herpes simplex* virus, can completely shut down host mRNA splicing machinery without affecting viral gene expression (Muhlemann et al. 2000; Lindberg et al. 2002). On the other hand, vaccinia virus and adenovirus rely on the host splicing machinery to process their genes (Yue et al. 1999; Huang et al. 2002). Conversely, mammalian hosts have been shown to use alternative transcripts to combat viral infection (Dinesh-Kumar et al. 2000; Fridborg et al. 2004). It is not currently known whether these mammalian infection responses also occur in insects.

In this study we have investigated the changes in *Drosophila* post-transcriptional RNA processing following infection by Flock House Virus (FHV). FHV is a positive strand RNA virus belonging to the *Nodaviridae* family and its RNA replication and virion assembly take place on the outer mitochondrial membrane of the infected cells (Ball et al. 1992; Miller et al. 2001). It can infect in several orders of insects, including Coleoptera, Lepidoptera, Diptera, and Hemiptera, as well as a nematode, plants, and yeast (Dasgupta et al. 2007).

We have analyzed splice variant transcripts of the *Drosophila melanogaster* 8-oxoguanine DNA glycosylase (*Ogg1*) gene following infection of macrophage-like *Drosophila* line 2 cells (DL2) with FHV. *Ogg1* is a DNA repair gene that is neither directly involved in host antiviral defense nor evidently useful to an RNA virus. It was selected to enable us to determine whether splicing instability is targeted to loci associated with viral infection and defense or whether its effects are broader. In this communication we report changes in the frequency and types of partially spliced *Ogg1* transcripts during FHV infection. We suggest that some of the splice variants observed during infection may be the result of infection-associated reduction in splicing efficiency.

Methods and Materials

Cell Line and Viral Infection

DL2 cells were obtained from ATCC, and cultured in Schneider's *Drosophila* media (SDM) supplemented with 9% heat inactivated fetal bovine serum and 100 µg/mL kanamycin (henceforth referred to as media) (Schneider 1972). DL2 cells were grown at 22°C without CO_2 and subcultured every 3 days. The cells were infected with Flock House Virus (FHV) (gift from Dr. David Miller) as follows: Subconfluent DL2 were gently dislodged and pelleted by centrifugation (300g). Cells were then resuspended in media and counted with a haemocytometer. Cell viability was determined with Trypan blue exclusion to ensure viability greater than 90%. Cell concentration was adjusted to 10^7 cells/ml with SDM and FHV added at a multiplicity of infection (MOI) of 10. Following inoculation cells were incubated at room temperature with occasional gentle mixing for 1 h. Cell density was then adjusted to 10^6 cells per ml with SDM, and incubated at 22°C without CO_2 exchange. A mock infection was performed on a replicate culture. The mock infected cells were treated as described above except the inoculum did not contain FHV. The cell morphology in all cultures was periodically monitored by light microscopy up to 72 h post infection. Under our infection protocol conditions, 95% of DL2 cells are infected by 8 h post inoculation and maximum viral RNA replication takes place between 4 and 16 h post inoculation (Dr. David Miller, pers. comm.).

RNA Extraction and RT-PCR

Total RNA was extracted according to the manufacturer's instructions using QIAGEN Easy RNA extraction kit from $2x10^6$ cells at 8, 12, 20 and 44 h post infection from the infected cells and at 8 h from the mock-infected control. The RNA was then treated with DNAse for 30 min at 37 °C followed by 1 h incubation at 80 °C to degrade genomic DNA. *Ogg1* was amplified from RNA using QIAGEN one step RT-PCR according to the manufacturer's instructions. The primers used were forward 5'- CGGGATCCAT GAAGGCTGTTTTAC and a reverse 5'-TAGATAAGAT CACTTTTTAGG.

Splice Variant Characterization and Frequency Calculation

PCR products were cloned into pGEM-T easy vector (Promega) and individual *Ogg1* transcripts were characterized by PCR, *Sal*I restriction endonuclease analysis, and DNA sequencing as previously described (Skandalis et al. 2004). Splice variant frequency was calculated as the fraction of the transcripts characterized that exhibited an alternative splicing pattern and it was reported as a percentage.

Results

Cell Morphology and Cytopathic Effect

FHV inoculated and mock infected DL2 cells were periodically monitored by light microscopy for morphological changes. DL2 cells adhered loosely to tissue culture plates during growth as previously observed (Schneider 1972). Adherence to culture plates decreased with time following FHV inoculation and gross morphological changes

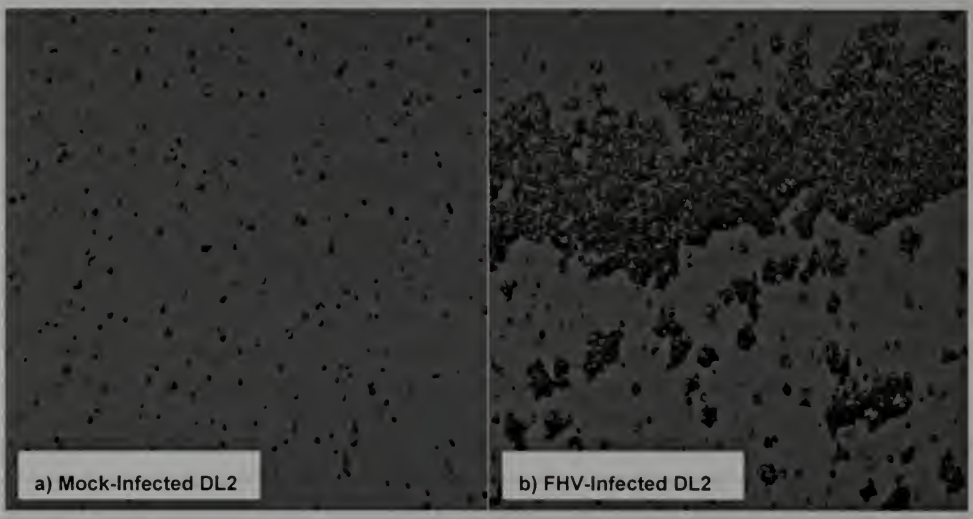

FIGURE 1. DL2 cell cultures 72h post infection (400x magnification). a) Mock-infected DL2 b) FHV-infected DL2.

were evident in the infected cells 72 h post inoculation (Fig. 1b), at which point the infected cells aggregated into a mass and became individually indistinct. By contrast the mock-infected control DL2 cell culture showed no differences in morphology or growth pattern compared to cells grown under normal conditions (Fig. 1a). The morphological changes of the infected cells were consistent with cytopathic cell death and no morphological changes associated with apoptosis were observed.

Cloning of *Ogg1*

The *Drosophila Ogg1* gene consists of 4 exons and 3 introns totaling 1476 nucleotides. The spliced *Ogg1* transcript is 1298 nucleotides in length. In our analysis of alternative *Ogg1* transcripts, the small size of the introns represented a potential problem since it was possible to amplify genomic *Ogg1* sequences directly from contaminating genomic DNA rather than from reverse transcribed mRNA. To avoid this problem, all RNA preparations were treated with DNAse to degrade any contaminating genomic DNA and then each RNA preparation was tested by PCR to confirm that it could not support *Ogg1* amplification without reverse transcribing mRNA (Fig. 2b).

Splice Variant Analysis

The effect of FHV infection on DL2 splicing was assessed at 8, 12, 20, and 44 hrs post FHV inoculation. Based on previous observations, approximately 95% of the cells are infected 8 h following inoculation with maximal viral RNA synthesis occurring approximately 20 h post inoculation. Amplified *Ogg1* transcript sequences were successfully obtained from each time point (Fig. 2a) and cloned into *E.coli* DH5α cells.

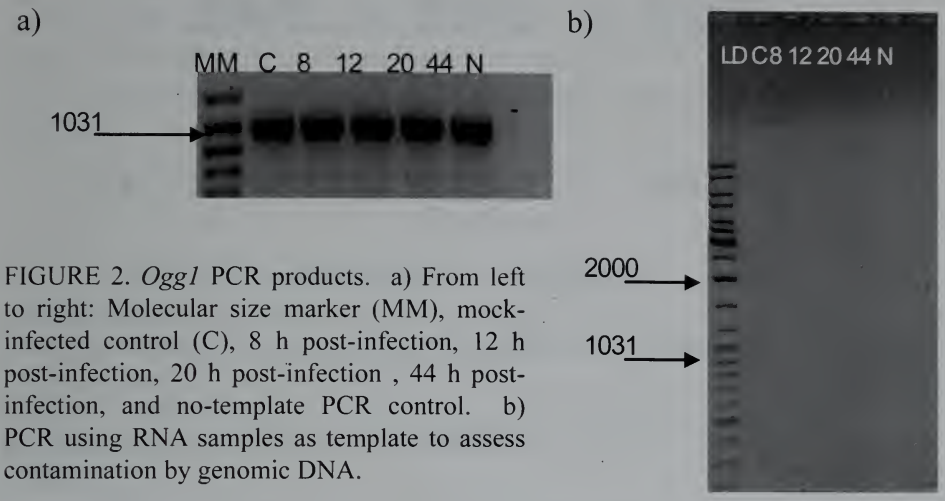

FIGURE 2. *Ogg1* PCR products. a) From left to right: Molecular size marker (MM), mock-infected control (C), 8 h post-infection, 12 h post-infection, 20 h post-infection , 44 h post-infection, and no-template PCR control. b) PCR using RNA samples as template to assess contamination by genomic DNA.

Individual cloned transcripts from each stage of infection were analyzed by restriction digest analysis and sequenced to determine the type and numbers of splice variants generated by *Ogg1*.

Overall, our analysis of *Ogg1* splice variants in *Drosophila* revealed five different transcripts (labeled SV1-5) in addition to the wild type (WT), all of which maintained some or all introns (Fig. 3). However, different combinations of splice variants were observed under different conditions. Characterization of 95 transcripts recovered from mock-infected DL2 revealed, in addition to WT, two types of splice variants: SV4, which retained introns 3 (frequency 1.1%) and SV2, which retained introns 2 and 3 (frequency 1.1%) (Fig. 4). Eight hours following FHV inoculation the frequency of splice variants increased to 15% (60 transcripts characterized). Of the three splice variants characterized, two were not detected in the control cells: SV1, which was completely un-spliced, retaining all introns (frequency 6.7%) and SV3 that retained introns one and two (frequency 3.3%). The third splice variant observed, SV4, was also present in the mock-infected control and at 8 hrs increased to 5%. Twelve hours post-infection the overall frequency of splice variants was less than at eight hours and similar to the frequency observed in uninfected cells (2.2 % of 95 transcripts characterized). The transcripts detected were SV4 and a novel variant, SV5, which retained intron 2. Twenty hours post-infection only one variant was detectable, SV5, with a frequency of 2.7% (N=74 transcripts). Finally, 44 hours post-infection the frequency of splice variants was elevated to 5.3% (N=95 transcripts) which was approximately double the frequency observed in the mock-infected cells but still 3-fold lower than the frequency observed 8 hours post-infection. The splice variants detected were SV1 (1.1%), SV2 (1.1%), SV3 (1.1%), and SV4 (2.1%) (Fig. 4).

Then frequencies of splice variants at 12 and 20 hrs were similar to the ones observed in mock-infected controls, but the types of variants differ (Fig. 4). Splicing of intron 2 exhibited a unique dependence on splicing of intron 1. In the hundreds of

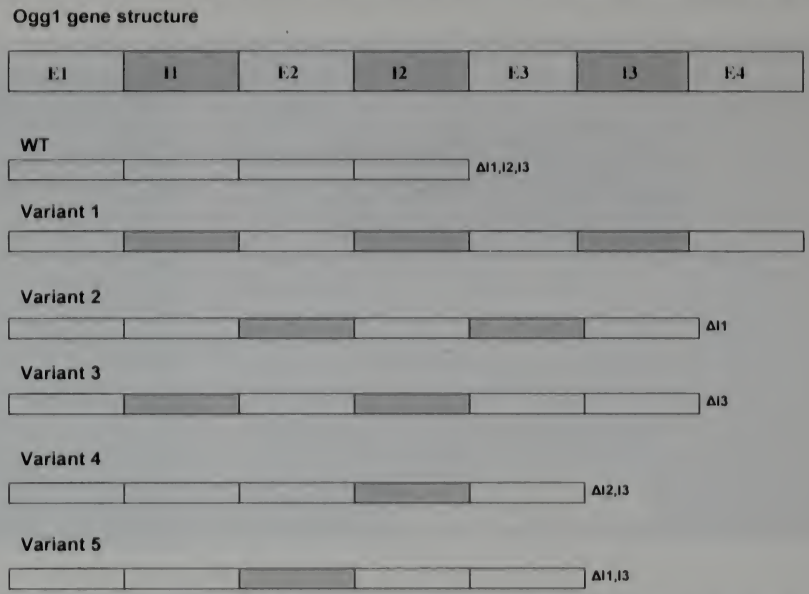

FIGURE 3. The structure of the *Drosophila Ogg1* gene and the types of splice variants detected in the infected and control DL2 cells. Not all variants were present in all conditions.

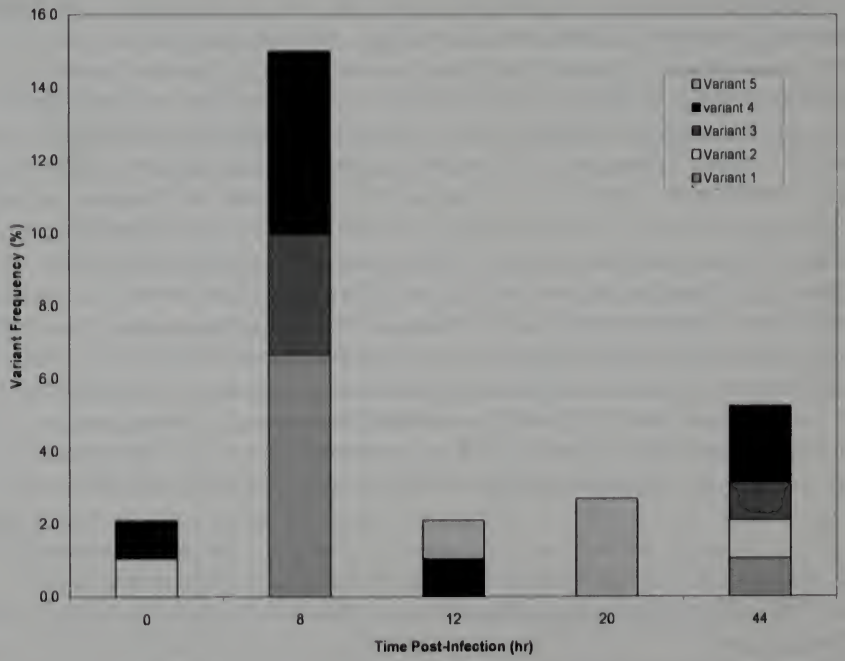

FIGURE 4. *Ogg1* splice variant frequencies pre and post infection in DL2 cultures. The mock infected control frequencies are indicated at time 0.

transcripts analyzed a variant maintaining intron 1 while intron 2 was spliced out was never observed. In other words splicing of intron 2 was predicated upon prior splicing of intron 1. By contrast variants were identified with intron 3 spliced out while introns 1 and 2 were retained indicating that prior splicing of intron 1 is not a prerequisite for intron 3 removal.

Discussion

Some mammalian DNA and RNA viruses, lacking introns, such as *Herpes simplex* virus can completely shut down host mRNA splicing machinery without affecting viral gene expression (Muhlemann et al. 2000; Lindberg et al. 2002). On the other hand, Vaccinia virus and Adenovirus rely on the host splicing machinery to process their genes (Yue et al. 1999; Huang et al. 2002). Conversely, mammalian hosts have shown to use alternative transcripts to combat viral infection (Dinesh-Kumar et al. 2000; Fridborg et al. 2004).

Here we analyzed splice variant transcripts of the *Drosophila* gene *Ogg1* following FHV infection of macrophage-like *Drosophila* DL2 cells. FHV does not itself undergo splicing, thus eliminating the possibility that the splicing machinery was manipulated for viral infection. *Drosophila* DL-2 cell lines were utilized since they exhibit macrophage-like genotypes, with receptors capable of detecting viral RNA and initiating native immune response (Shields et al. 1975; Rehli et al. 2003).

All *Ogg1* splice variants identified in this study maintained some or all introns. Since transcripts with un-excised introns were observed in the FHV infected as well as in the mock-infected control cells it is unlikely that the transcripts represented infection-related disregulation of splicing. These observations indicated that the transcripts detected in *Drosophila* cells were not genuine alternative transcripts but rather immature transcripts awaiting further splicing. Even though splicing is normally co-transcriptional, splicing can occur post-transcriptionally (Aguilera 2005). In the control, non-infected cells, the intron retention pattern of the splice variants (retention of intron 3 or introns 2 and 3) was consistent with sequential removal of introns. However, in the FHV infected cells intron removal was not sequential, with some transcripts retaining intron 2 or, introns 1 and 2. Unspliced transcripts were also detected indicating that following infection splicing is not co-transcriptional.

Overall, splicing was adversely affected by FHV infection in *Drosophila* DL2 cells. Following FHV inoculation, we observed an accumulation of immature *Ogg1* transcripts suggesting a retardation of host gene splicing. This observation and the shift in the types of transcripts observed during infection implicate changes in the regulation or localization of some splicing factors as the likely mechanism responsible. These changes were likely host-directed since the simple FHV lacks the molecular machinery to implement changes in splicing.

The results support the conclusion that viral infection may be accompanied by host-produced splicing instability, which has the potential to generate novel splice variants not associated with the generation of useful splice variants for either the host or the infecting virus. Nonetheless, this instability has the potential of degrading the

transmission of genetic information of both the host and the virus. In mammalian systems, it appears that splicing of host genes disrupts viral infection. *Herpes simplex* virus, vaccinia virus and adenovirus decrease splicing of cellular pre-mRNAs by inducing hypo-phosphorylation of the human ASF/SF2 protein, a member of the evolutionary conserved SR family of splicing factors (Kanopka et al. 1996; Huang et al. 2002; Sciabica et al. 2003). Differential expression of several host splicing factors was also observed following cytomegalovirus infection (Adair et al. 2004). Rous sarcoma virus (RSV) contains a negative regulator of viral splicing, and is has been hypothesized that the suppression of splicing benefits the virus by increasing the pool of unspliced viral RNA that will be packaged as progeny genome (Maciolek and McNally 2007).

To our knowledge this is the first report that infection may be accompanied by a general splicing instability in *Drosophila*. Given the similarities in immune responses between mammals and *Drosophila*, reviewed in (Muller et al. 2008) it may have far reaching implications about metazoan defenses against infection. It would be a great interest for infection treatment to determine in future experiments whether this phenomenon constitutes an adaptive defensive strategy by the host or simply a by-product of other anti-viral responses.

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MORPHOLOGICAL AND DNA SEQUENCE DELINEATION OF TWO PROBLEMATIC SPECIES OF *CERATINA* (HYMENOPTERA: APIDAE) FROM EASTERN CANADA

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Abstract

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Ceratina are small, twig-nesting carpenter bees of cosmopolitan distribution. In Eastern Canada, *C. calcarata* and *C. dupla* live in sympatry, and females are so similar morphologically that many previous species lists combined both taxa into one category. The problem is that the description of the traditional morphological character used to distinguish between them is easily misinterpreted, placing emphasis on puncture abundance rather than distribution. In this paper we propose a clearer description of this character and provide confirmation using scanning electron microscopy to confirm that puncture placement takes precedence over abundance. We tested the feasibility of the new character for distinguishing between these two species, by testing it on non-entomologists, who used it with 87% accuracy. Moreover, DNA sequencing of the cytochrome oxidase subunit 1 gene for the same specimens revealed 1.29% sequence divergence between the two morphs, providing additional support that *C. calcarata* and *C. dupla* are indeed distinct species that can be distinguished morphologically.

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Introduction

Ceratina are small, often abundant, carpenter bees that nest in pithy cores of dead broken stems and twigs (Michener 1985). Although they are found across the globe, they have rarely been studied in detail, and much remains unknown about their basic biology and behaviour (Michener 1979). The genus comprises 19 subgenera, of which three are found in North America (Michener 2000). In eastern Canada, from Ontario to the Maritimes, only three species are found, all members of the subgenus Zadontomerus: *Ceratina calcarata* Robertson, *C. dupla* Say, and *C. strenua* Smith (Daly 1973). These three species are also found throughout the eastern United States (Daly 1973). Although all three species often occur in sympatry in more southerly parts of their range, *C. strenua* is rarely found in Canada and southern Ontario is likely the northern edge of its range. However, in most of

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eastern Canada, *Ceratina calcarata* and *C. dupla* are sympatric.

Morphologically, *C. strenua* is easily distinguishable from *C. dupla* and *C. calcarata*, most notably by its smaller size. However, separating *C. dupla* and *C. calcarata* is problematic: although there are recognizable differences among males, females are remarkably similar. As a result, many entomologists have given up trying to differentiate female *C. calcarata* and *C. dupla* in their samples and instead combine both taxa into one category for their species lists (Clinebell 2002; Mitchell 2002; Reed 1995). Although it is likely that both species occur in sympatry throughout much or all of their range, it is difficult to discern whether one species may predominate in an area as floral records and geographic distributions rely on males of each species (Daly 1973). The inability to differentiate females of these problematic species has also negatively affected studies on their life history. For example, Johnson's (1988) study on *Ceratina calcarata* sex ratios in Indiana, omitted all-female broods because he was only confident in identifying males of the species.

Currently, eastern North American *Ceratina* are identified using Mitchell (1962). In this key, females of *C. dupla* and *C. calcarata* are distinguished by comparison of the number of punctures in the area of the scutum between the notaulices: *C. dupla* have numerous punctures whereas *C. calcarata* have few, if any. The problem is that this character description is easily misinterpreted, and there is disagreement about whether the distinguishing character works. However, this taxonomic problem is difficult to resolve without independent evidence that the specimens being examined are indeed correctly identified members of the two species.

In this paper, we propose a simpler description of the traditional character used to distinguish female *C. calcarata* and *C. dupla*. Following thorough examination and description of the character based on scanning electron microscopy (SEM), we test this character's reliability by assessing non-entomologists' ability to use it to identify the two species. In addition, we provide genetic evidence that *C. calcarata* and *C. dupla* are indeed separate species based on comparisons of their DNA sequences for the cytochrome oxidase subunit 1 gene for the two species, allowing us to independently distinguish the species identities of specimens used to validate our proposed taxonomic character.

Methods

Sampling and Identification

Specimens were collected in pan traps during the summers of 2005 and 2006 from St. Catharines, Ontario. Pan traps used were blue, white and yellow plastic bowls (6 oz; SOLO PS6-0099) filled with soapy water and spaced ten metres apart in transects. Insects land on the water surface and drown, as the soap in the water acts as a surfactant. All specimens were subsequently preserved in 70% ethanol. *Ceratina* specimens were initially identified using Mitchell (1962). Following identification, most samples were returned to 70% ethanol for storage, but 24 specimens (12 females from each species) were dissected for SEM imaging and DNA extraction (see below). With legs and wings removed, the thoraces of these specimens were used for SEM imagery of the dorsal side, the heads and abdomens being reserved for DNA.

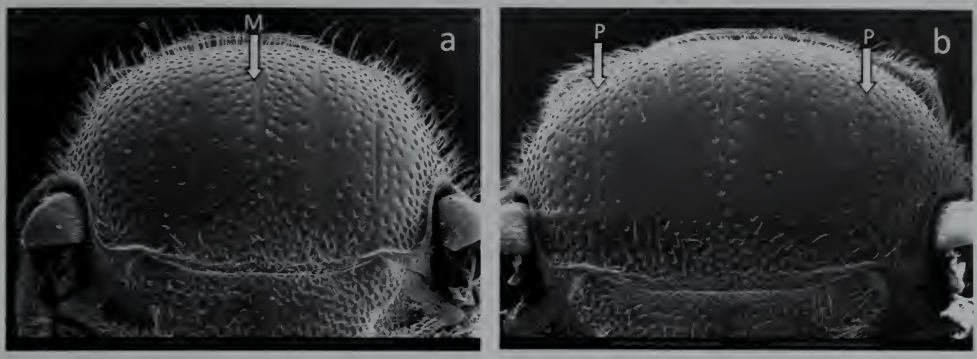

FIGURE 1. SEM images of female *Ceratina* thoraces. a. *C. dupla*, more punctate with two or more complete longitudinal rows of punctures inside the parapsidal lines and around the medial line. Arrow indicates the medial line (M). b. *C. calcarata*, less punctate with one to two incomplete longitudinal rows of punctures inside the parapsidal lines and around the medial line. Arrow indicates the parapsidal line (P).

SEM images were obtained using an AMRAY 1600 Turbo scanning electron microscope. Specimens were mounted onto a carbon adhesive tab and silver paint was applied to the specimen edges to aid in sample conductivity. Using secondary electron scintillation detector and 15kV accelerating voltage, images were processed using ORION Digital Image grabbing software (version 6.51). SEM images were used to confirm the validity and consistency of the revised morphological character developed to distinguish between female *C. calcarata* and *C. dupla*. SEM images and type specimens have been retained in the collection of M.H. Richards at Brock University.

Taxonomy Test for Non-Taxonomists

Since Mitchell's (1962) key is sufficient to identify male *C. calcarata* versus *C. dupla* and *C. strenua* of both sexes these characters were not altered from the original key descriptions. However, due to the difficulty distinguishing female *C. calcarata* versus *C. dupla* a new character was developed to differentiate females of the two species. Following scanning electron microscopy, SEM photographs of female *C. calcarata* and *C. dupla* were given to 24 naive non-entomologists along with 15 pinned specimens of *C. calcarata* and 13 pinned *C. dupla*. Bees were randomly labeled from 1 to 28 and placed in a box. Identifiers were given the box and a dissecting microscope and shown where on the bee the scutum was located (most volunteers had never seen a bee under a microscope before). Identifiers then assigned each specimen to either *C. calcarata* or *C. dupla* using figures 1 and 5, as well as by reference to a written description of the character (key to females, couplet 2). Identifiers were later graded against the original identifications by the senior author.

DNA Sequence Analysis

Total genomic DNA was isolated from the head and abdominal segments of the 24 SEM specimens, using the Sigma-Aldrich GenElute Mammalian Genomic DNA

Purification Kit. Manufacturer's instructions were followed with one additional step, in which an extra centrifugation was incorporated after proteinase K digestion to remove bee fragments that might clog the spin columns. Portions of the mitochondrial gene COI were amplified using primers mtd-8 and 12 (Simon et al. 1994; University of British Columbia Biotechnology Laboratory, Vancouver) in 20 µl reactions with 2.5 mM MgCl2, 200 µM dNTPs, 2.0 µM each primer, 0.25 U Sigma Jumpstart Taq polymerase, and 2 µl of Taq reaction buffer (supplied with the enzyme). PCR reactions were as follows: initial heating to 94°C for 5 min, followed by 30 repetitions of 94°C for 1 min, 54°C for 1 min, 72°C for 1 min, and final extension at 72°C for 5min. Amplification products were purified by ethanol precipitation and analyzed on 0.8% agarose gels containing 10mg/ml ethidium bromide. DNA sequencing was carried out at McGill University and Genome Quebec Innovation Centre in Montreal, using the same primers. Sequences were edited using BIOEDIT (Hall 1999) and aligned using CLUSTAL (Thompson et al. 1994) using default settings with the exception of gap open penalties increased to 50. All sequences have been deposited in GenBank under accession numbers EF534228-EF534247. We used Analysis of Molecular Variance (AMOVA) as implemented in Arlequin 3.11 (Excoffier et al. 2005) to compare genetic variation within and among specimens identified with the revised morphological character.

Results

Morphological Differences

Using light microscopy and SEM images in combination with corresponding COI sequences revealed the subtle yet consistent dimorphism between these species. *Ceratina calcarata* are less punctate than *C. dupla*, just as Mitchell (1962) describes, but the placement of punctures around the parapsidal and medial lines is better at differentiation than their abundance (key to females, couplet 2). This difference was confirmed by an 87% correct identification rate among the non-entomologists tested.

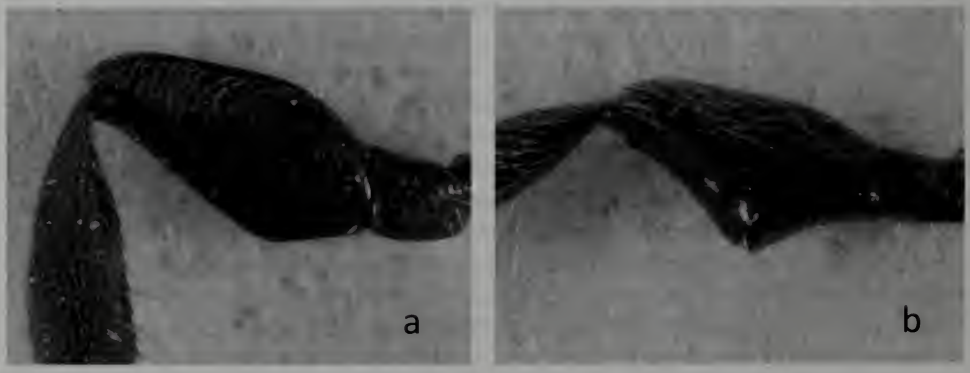

FIGURE 2. Hind femurs of male *Ceratina*. a. *C. dupla,* hind femur somewhat dilated toward base, but without a median projection. b. *C. calcarata*, hind femur with a median,

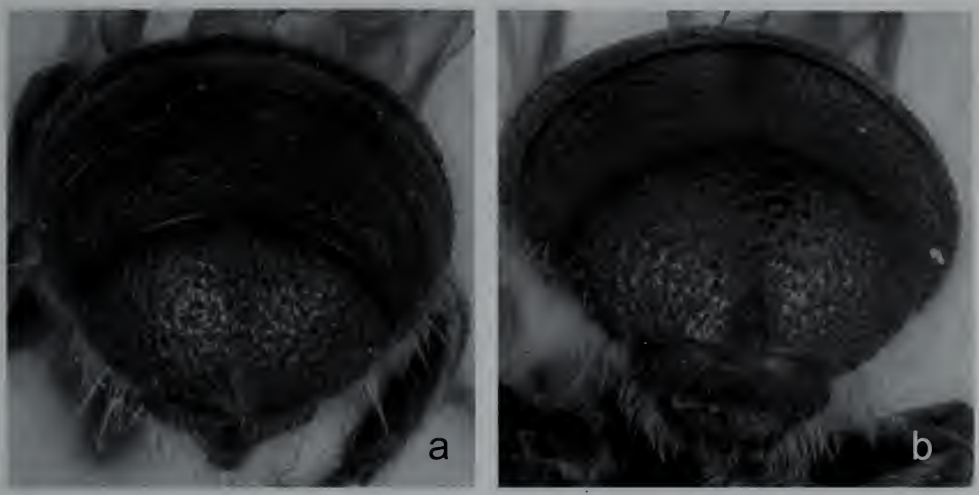

FIGURE 3. Tergum 7 of male *Ceratina*. a. *C. strenua,* carina of tergum 7 very narrow. b. *C. calcarata*, carina of tergum 7 broad.

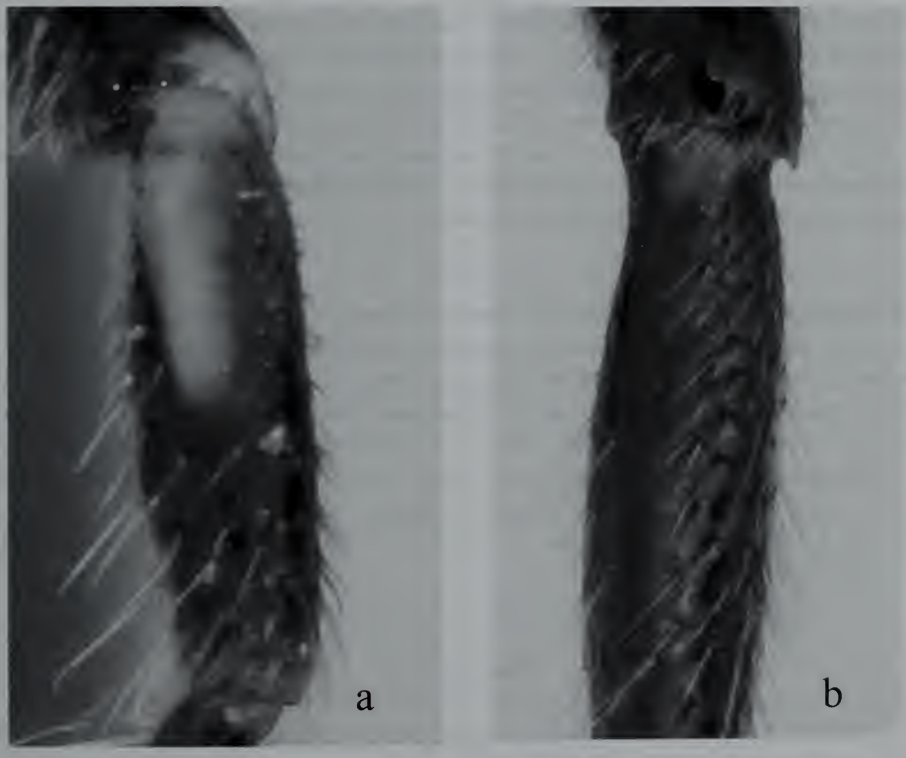

FIGURE 4. Front tibia of female *Ceratina.* a. *C. strenua*, front tibia with a basal ivory stripe. b. *C. calcarata*, front tibiae with at most a basal ivory spot.

FIGURE 5. Thoracic puncture dimorphism of female *Ceratina*. a. *C. dupla*, more punctate with two or more complete longitudinal rows of punctures inside the parapsidal lines and around the medial line; central area between medial line and parapsidal lines always punctate in posterior half of mesoscutum. b. *C. calcarata*, less punctate with one to two incomplete longitudinal rows of punctures inside the parapsidal lines and around the medial line; areas between medial line and parapsidal lines usually impunctate with, at most, a couple of central punctures in posterior half of mesoscutum.

DNA Sequence Divergence Between *Ceratina calcarata* and *Ceratina dupla*

We compared 774 base pairs of cytochrome oxidase one (COI) for 11 individuals of *C. calcarata* and 9 of *C. dupla* that had been previously identified using the morphological character described above. Sequences fell cleanly into two groups, distinguished by 7 fixed differences between the two species, providing evidence that *C. calcarata* and *C. dupla* are indeed genetically isolated in sympatry. Pair-wise comparisons among all individuals revealed significantly greater sequence divergence between species (mean pairwise difference, 1.29%) than within species (*C. calcarata*, 0.46 %; *C. dupla*, 0.029 %; AMOVA: $F_{ST} = 0.80993$, df= 1,18, $p<0.00001$).

Discussion

The biological species concept defines species as groups of actually or potentially interbreeding natural populations that are reproductively isolated from other such groups (Mayr 1942). Previous studies have provided some evidence that *C. calcarata* and *C. dupla* are separate species due to a lack of heterozygotes at multiple isozyme loci in natural populations (Hung & Norden 1987). Our study confirms that the two species are indeed

genetically as well as morphologically distinct. Although hybridization is thought to be possible in artificial environments such as greenhouses and flight cages (Hung & Norden 1987), the fact that these sympatric species are genetically distinct suggests differences in life history and behaviour are possible.

Limited research has been done on the life history and behaviour of natural populations of *Ceratina* in North America. Grothaus (1962) found that *C. calcarata* prefer to nest in open areas, usually in older, drier stems, while *C. dupla* prefer shaded areas and recently dead stems. This life history difference between sister species parallels a remarkably similar finding in two Asian species of the subgenus *Ceratinidia, Ceratina flavipes* and *C. japonica*, which are sympatric throughout Japan. Females of *C. japonica* prefer to nest in bushes or sparsely wooded areas, remaining shaded and humid, whereas *C. flavipes* nest in comparatively open, dry areas (Sakagami & Maeta 1977). As with *C. calcarata* and *C. dupla*, males of the two Japanese species are quite distinct, but females are very difficult to distinguish (Shiokawa 1963; Yasumatsu & Hirashima 1969).

Previous studies have shown COI to be a useful indicator gene to differentiate even morphologically indistinguishable sister species. *Halictus ligatus* and *H. poeyi* are sympatric, eusocial species with slightly different phenologies (Dunn et al. 1998). They exhibit approximately 0.4% COI sequence variation within species and 4 to 5% between species (Danforth et al. 1998). The degree of divergence between these two *Halictus*, is comparable to that between the aforementioned *C. flavipes* (Genbank accession no. AY250190) and *C. japonica* (AY250192) (Cronin 2004); comparison between these two sequences reveals about 4.1% divergence. Both these species pairs are considerably more divergent than *C. calcarata* and *C. dupla* appear to be.

Morphologically similar females are found in various Old World subgenera of *Ceratina*, including *Ceratinidia* (Shiokawa 1963; Yasumatsu & Hirashima 1969), *Ceratina sensu stricto* and *Neoceratina* (Hirashima 1971), and numerous New World *Zadontomerus* (Daly 1973). Although males of this genus have proven simple to distinguish based on femoral tooth projections, tergal processes, elaborate genitalia and sternal suture modifications, it seems that females across the genus *Ceratina* have more understated distinctions. Eventually, closer examination for subtle differences among sympatric species, perhaps aided by molecular systematics approaches, may reveal consistent female key characters across the genus.

Key to species

Males

1. Hind femur somewhat dilated toward base, but without a median projection, the greatest width near the base no more than a third its length (Figure 2a)..... .....***C. dupla*** Say
– Hind femur with a median, triangular projection, femoral width at this point about equal to half its length (Figure 2b) 2
2. Carina of tergum 7 very narrow, fully as long as broad, and not over a fourth as broad as the tergum (Figure 3a).....***C. strenua*** Smith
– Carina of tergum 7 at least twice as broad as long, fully half as broad as width of the tergum (Figure 3b) .....***C. calcarata*** Robertson

Females

1. Front tibia with a basal ivory stripe; small (body length 5-6 mm) (Figure 4a)..... .....*C. strenua* Smith
– Front tibia with at most a basal ivory spot; usually larger (body length 7 mm or more) (Figure 4b) 2
2. One to two complete and up to four incomplete longitudinal rows of punctures inside parapsidal lines and around medial line; area between medial line and parapsidal lines always punctate in posterior half of mesoscutum (Figures 1a and 5a)..... ..... *C. dupla* Say
– One to two incomplete longitudinal rows of punctures inside the parapsidal lines and on either side of the medial line; areas between medial line and parapsidal lines usually impunctate with, at most, a couple of central punctures in posterior half of mesoscutum (Figures 1b, 5b).....*C. calcarata* Robertson

Acknowledgments

The assistance of Mark Frampton was invaluable in both the field and the lab. We thank Doug Currie and Chris Darling for allowing us to use the Royal Ontario Museum's microscope imaging system, Glenda Hooper for producing the SEM images, and all those volunteers who cheerfully tested the key. This study was supported by research funding from NSERC and Brock University to MHR, and by NSERC Undergraduate Summer Research and Brock University Student Research Awards to SMR.

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BOOK REVIEW

Insects: Their Natural History and Diversity. ***by*** **S.A. Marshall. Firefly Books Ltd. Richmond Hill, Canada. 720 pp. ISBN-13: 978-1-55297-900-6; ISBN-10: 1-55297-900-8 (Hard cover: 2006). $ 95.00 CAN.**

Over the course of the last two years this 'big green book' has become my favourite (and most often used) entomological reference. With its interestingly written text and outstanding photographs, this comprehensive and user friendly book is a must have for professionals and amateurs alike.

Although large, *Insects: Their Natural History and Diversity* is far from intimidating. The introduction opens with a basic explanation of insect anatomy and biology, and the reader is instructed to "get your hands on a grasshopper" and follow along as the text explains the different body segments. The chapters that follow are organized either by insect order or in logical groups such as "The Wingless Insects (Springtails, Diplurans and Bristletails)." Also included is a valuable chapter on methods of collecting, keeping and photographing insects, as well as a fantastic picture key to the insect families.

Chapters in the main body of the book are organized into two sections. The first comprises brief biological overviews of the insect families or superfamilies found in each chapter. This introduces the reader to the fascinating biology and behaviour of a particular group of insects. In these introductions Marshall gives the reader insight into the most interesting and unusual aspects of that family. It reminded me of early mornings as an undergraduate at the University of Guelph, when an 8:30AM class seemed more like story time than a lecture (please note that Steve has not commissioned me to say nice things about his lectures!) The second portion of each chapter provides spectacular photographs of many of the species representing each family discussed. Almost all of the photographs are of the insect in nature, quite often engaging in one of the interesting behaviours discussed in the introductory section of the chapter. Captions for each photo contain at least the genus (most often species), as well as an explanation of the photograph.

The last section of *Insects* contains the picture keys. Again designed with the amateur entomologist in mind, the illustrations eliminate the need for specific taxonomic knowledge; and most of the characters used can be seen without a microscope. The first two keys take the reader down to order, while further, more specific keys help the reader identify their insect to family. Picture keys of common immature insects can also be found in this section. Once a family is reached a page number is given; the reader can then refer to that section of the book to look at photographs of individuals from that family. I have found that this is often a great way of affirming my original decision about a specimens identity.

I have completely lost count of how many times and in how many ways *Insects* has been useful. Aside from being a fantastic way to get your friends interested in entomology (by showing them stunning photographs of bizarre and beautiful insects), I can honestly say that I have picked up this book at least twice a week for the last year or so, either to identify an insect that I have found in the field, or to look at as I would a coffee table book. At $95.00 it is well worth the investment and is an absolutely essential component of any entomological library!

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IN MEMORIAM

Edward Coulton Becker
1923 - 2008

Dr. Edward Becker

Dr. Ed Becker, Fellow of the Entomological Society of Ontario, passed away on May 13, 2008 at the age of 85. He was a research scientist at Agriculture and Agri-Food Canada from 1952-1980, working as a taxonomist at the Canadian National Collection (CNC) of Insects, Arachnids and Nematodes in Ottawa. His area of expertise was the systematics of click beetles (Coleoptera: Elateridae), which include many Canadian crop pest species. Following retirement, he became an honourary research associate at the CNC and continued to come into work nearly every day for the past 28 years.

Ed was born on March 15, 1923 in St. Louis, Missouri to Coulton and Grace Becker and was the first of six children, and a twin. He spent his early years on the family farm near Williamsville MO. After high school, he attended the University of Missouri where he took three years of agricultural studies before joining the US Marine Corps. After the war, Ed met Martha Mae Elliott at a church camp at Lake of the Ozarks and they were married in 1948. The newly married couple soon moved to Honduras, where Ed worked as an entomologist for the Standard Fruit Co. They returned and Ed attended the University of Illinois, earning his PhD in entomology in 1952 with a thesis on the taxonomy of *Agriotes* (Coleoptera: Elateridae). By good fortune, Agriculture Canada was looking for a taxonomist to work on click beetles at the CNC and he and Martha soon moved to Ottawa.

Among his 36 systematics publications are monographic revisions of the large and economically important click beetle genera *Agriotes* and *Athous* (Coleoptera: Elateridae) of North America. This work was central to controlling a major North American crop pest problem. Altogether, his work has been world-wide in scope, spans several beetle families, and includes descriptions of 53 new species and two new genera. Towards the end of his research career, Ed co-authored a series of major scientific articles with the Japanese scientist Hitoo Ôhira. Becker's systematic research was innovative, in that he wrote the most rigorous, detailed descriptions and keys for Elateridae to date and pioneered new unexplored morphological character systems.

In addition to Ed's research, he was active in many entomological societies and organizations, working as treasurer of the Entomological Society of Canada (1961-1985), Section A representative and governing board member of the Entomological Society of America (1982-1984), Editor of *The Coleopterists Bulletin* (1983-1990) and President of the Coleopterists Society (1971-1972). Perhaps his biggest contribution to entomology was through the CanaColl Foundation, a non-profit organization that Ed helped create and almost single-handedly nurtured for the past 36 years. The foundation promotes taxonomic

research at the CNC by providing funds to visiting entomologists who curate the collection. In addition, Ed wrote and distributed a quarterly newsletter for retired entomologists and their spouses for the past 18 years. For many years, he also visited public school classrooms to promote entomology, often with a tarantula named Carmen at his side. Ed was recognized for his work by receiving the Queen's Silver Jubilee Medal (1978), the Canada Commemorative Award (1984). He was made a Fellow of the Entomological Society of Canada (1974) and an Honourary Member of the Entomological Society of America (1997), at one time, the only person to be so honoured by both societies, and was also made a Fellow of the Entomological Society of Ontario (2003). Following retirement, he became an honourary research associate at the CNC and continued to come into work nearly every day for the past 28 years. His tireless efforts to promote entomology and the CNC have had far-reaching effects not only in Canada, but throughout the world. He was very well known in the entomological community, partly because of his friendly, social nature, but also because he attended every single ESC meeting for the past 49 years! Ed's cheerful, positive attitude, sense of humour and love of bowties will be sadly missed.

Donations in Ed's memory can be made to the CanaColl Foundation, c/o Andy Bennett, 960 Carling Ave., Ottawa, ON, K1A 0C6.

ANDY BENNETT
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and

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